

13 November 1980

How to speak out on Sakharov *et al.*?

Gor'kii, where Andrei Sakharov is under house arrest, is well to the east of Moscow. Those who live there, willingly or otherwise, cannot at this time of the year forget that the long winter has begun. The sun will not shine strongly again until the best part of half a year has passed. The central isolation from which the inhabitants of Gor'kii suffer even at the best times of the year will for a long time to come be indistinguishable from desolation. Andrei Sakharov's letter (see page 112) is thus a reminder of the plight of others than himself. In some respects it may be as bad to have to live in Gor'kii because nothing else suggests itself, as to happen to live in Gor'kii because one has been taken there by force. If imprisonment is the lack of freedom, and if the sense of freedom is the sense of having chosen to live in a chosen way, many of Sakharov's fellow residents may be as deprived of liberty as he without question is. Sakharov should not blame himself if his letter prompts those who read it to remember that his problem is part of a larger problem, one that will still be with us in the spring. How could it be otherwise, in the week that the first review conference on the Helsinki accords begins in Madrid?

Sakharov makes several points, some of them particular. Plainly he is dismayed that the conference at the Hague at the beginning of September should have been based on assumptions that are incorrect. The KGB may have tried to prevent him publishing his three recent papers in English in the West, but it had no influence with the editors of *Zhurnal éksperimental'noi i teoreticheskoi fiziki*. There is no scientific library across the street, only "mud and piles of rubbish". (No doubt the snow has by now made them less unsightly.) The reason that he cannot talk to scientific colleagues is not that the others who work in Gor'kii work on classified projects but that he is prevented from going out, and from receiving visitors. (The personalized radio jamming equipment is a refinement.) There is no telephone.

Sakharov also raises more general questions. Why, he asks, is it supposed that his treatment is mild simply because he has not been sent to a labour camp? Is loss of liberty now measured on a discontinuous spectrum, with hardly anything between full citizenship and endangered life? If so, he implies, any loss of liberty is absolute. To know that the KGB can break into your flat when it chooses may be as intolerable as to know that you may be worked to death on somebody else's whim. Naturally he is sorry for his friends and fellow-scientists — Orlov, Shcharanskii, Velikanova, Nekipelov, Lavut, Ternovskii, Osipova and the rest. The severity of their plight does not make his own supportable.

Imprisonment without trial

Then, he asks, what happens when a person's loss of liberty comes about because a government has assumed the right to break its own laws? The constitution of the Soviet Union resembles that of other modern states in its assurance that people will not be deprived of liberty without due process, usually a trial by a court. So, Sakharov asks, how has the word got about that it can be in his interest not to make too much fuss outside the Soviet Union in the hope that the authorities will then quietly relent and let their illicitly imprisoned prisoner return to Moscow? What Sakharov wants, he says, is due process — a formal charge, a fair trial and either a fair sentence or a discharge.

Sakharov appears not to appreciate the enormity of his demand. The Russians (which is not necessarily a synonym for "the Soviets") have had a flair in the past few centuries for mingling tyranny with essays at rebellion. The one, of course, provokes the other. Tolstói might have been as complacent as his fellow-landowners if the feudal state of the nineteenth century

had been less repressive. Dostoevskii might have consented to entertain the middle classes with popular romances if only there had been more romance to brood about. Sakharov is one of the many who, in the past few years, have dug in their heels and insisted on the rule of law and the validity of international agreements such as the Helsinki accords. By doing so, he runs great risks for himself and his family. Forced emigration would solve nothing, but would be the ultimate humiliation. Sakharov's plight is haunting.

What, if anything, can be done to help? Sakharov's letter provides some clues. He is concerned about his relatives and about his son's fiancée. Experience in the past few years has shown that it may sometimes help if the names of those threatened with trouble of some kind are well known outside the Soviet Union. But publicity is not a certain remedy, as the cases of the Orlovs of that world demonstrate. Perhaps the best that can be offered is the occasional message of good cheer, perhaps even a reprint of a scientific paper that might interest him. It is sad that there is so little else to offer, with the winter setting in at Gor'kii.

But is this not an issue on which the interests of the scientific community as a whole should be engaged? The question is unfortunately more complicated than it seems. Sakharov may be a member of the scientific community, but he is also a citizen of the Soviet Union, and his disagreement with his government is about civil liberties, not scientific issues. To what extent, in these circumstances, is the scientific community entitled to be engaged?

Constraints on protest

To a first approximation the answer is that the international scientific community has no special duty to protest at what is happening to Sakharov and those of his fellow scientists who are formally imprisoned. The Soviet Union has a constitution which guarantees due process, but also a criminal code which arranges that people are brought to trial for reasons that are elsewhere unacceptable. The trouble is that the scientific community as such has no special competence in persuading national governments to follow seemingly legal practices. And even where Soviet citizens such as Sakharov have been harmed outside the law, by being deprived of jobs or deprived of liberty without even an unfair trial, to a first approximation the issue is not one for the scientific community. Others than scientists are dealt with in such ways. Injustice, it appears, is impartial. This is why, in the past several years, the problems of those such as Sakharov have been such a rich source of frustration for their colleagues elsewhere.

The second approximation, fortunately, offers a little more hope, and for two reasons. Scientists are fond of saying that science is international and that the scientific community is inherently more interdependent than other communities of scholarly or professional people. Too much is often made of these propositions, which, nevertheless, contain a germ of truth. We are all the losers if people like Sakharov cannot work as effectively as they might, and talk as freely as they would wish about what they have done. Ironically, the past few decades have demonstrated the eagerness of even the Soviet government to make formal arrangements for the movement of Soviet scientists in and out of the Soviet Union.

The second chink of hope is that the Helsinki accords exist. They are not a formal international convention, but a declaration of intent. The declared intention is liberal, if only vaguely so. People are to be free to move from one country to another if they wish, free to correspond with colleagues elsewhere, free to do a variety of things now often denied. The objective of the



conference opening this week in Madrid is to assess progress and to decide what should next be done. The outcome will not carry the Helsinki accords much further. The most immediate danger is that such hard things will be said that the process of relaxation will be set back. Yet the accords provide a licence for scientists outside the Soviet Union to speak their minds from time to time.

This is the spirit in which more than 270 distinguished scientists bought advertisement space in the London *Times* last week asking for the release of fellow scientists now locked up. Lord Todd, the President of the Royal Society, followed this on Friday with a letter in *The Times* asking that the British government should make an issue with the Soviets about Sakharov, Orlov, Shcharanskii and the rest. These developments are creditable. The US National Academy of Sciences declared itself to the same effect more than a year ago. But it is important that the scientific community in the West should clearly appreciate what it is about with such declarations. Because of its connections, because science is indeed international, it happens to have learned of some appalling injustice. But classicists, farmers and lorry drivers are similarly deprived of liberty without good cause. The scientific community must appreciate that by asking that Western governments should do what they can to persuade the Soviet government to change its ways, it is asking a political and not a scientific favour. The scientific community is entirely within its rights to do so. Science will continue to be diminished if the Soviet contribution is inhibited by illiberality. The knowledge that protest is bound to be understood as political protest has no doubt helped to keep the scientific community silent all these years. To urge governments to take up cudgels is the proper course.

No boycott

Talk of boycotting relations with Soviet scientists is, however, mistaken. In the past few weeks, Sir Brian Pippard and others have been drumming up support for a temporary boycott. Scientists in the West, they say, should not receive Soviet scientists in their laboratories and should not attend conferences in the Soviet Union until the diplomatic conference in Madrid is over. The argument would make sense if there were reasons to suppose that a boycott would succeed in influencing what happens at Madrid. The chances of that are small.

Although there will be many in the West who will now follow their individual inclinations and boycott Soviet science, it will be best if those not that way inclined can try to strengthen relationships with their Soviet colleagues, at the same time leaving them in no doubt of how the illiberality of the Soviet state offends the consciences of others and impedes the advancement of science as well. Soviet colleagues should be welcomed, if anything more warmly than in the past, but also spoken to more openly than has been the custom. The genteel convention that one does not embarrass a visitor by talking politics is now outmoded. For the best but cruel hope for Sakharov and his like is that there should be more of them.

In following such a line stridency must be avoided. A sense of perspective is essential. It is thus relevant that what the Soviet government has done to Sakharov *et al.* does not hold a candle to, say, what Pol Pot did in Cambodia between 1976 and 1978. Moreover, the West cannot be too smug in its complaints against the Soviet way of dealing with people such as Sakharov — it is after all only a quarter of a century since Senator Joseph McCarthy was riding high in the United States administering injustice in an arbitrary way. Russian illiberality is, however, a special challenge because the Russian state is within an ace of being decent. Personal freedom is guaranteed by the constitution. The Soviet educational system, much admired elsewhere, creates expectations of freedom. The industrial development of the past half century depends on the involvement of trained people in a variety of occupations, technical and managerial, that are efficiently carried out only when people are free to move about within the system and free to contribute to industrial development in ways that best suit individual talents. Is it too much to hope that some future Soviet government will recognize that economic and industrial stagnation is too high a price to pay for the right to lock

people up on flimsy charges?

The revolution, ostensibly directed at Tsarist feudalism, seems not have touched what is the essence of feudalism — the notion that people's lives belong in their entirety to the state. This is the basis on which it is supposed that ordinary unrebelling people in the Soviet Union should have less freedom than is habitual elsewhere to choose what they work at and where they live. The institution of the collective farm is not very different from that of the mediaeval *desmesne*, while the recent understanding that members of the collectives should be allowed to work some land on their own account is the Soviet analogue of the mediaeval practice of granting the right to work strips of land in a common field to the dutiful dependants of a manor.

Experience of the Dark Ages in Western Europe shows clearly enough that in such a feudal system the lack of freedom is not absolute and the condition of society not entirely static. Just as, in mediaeval society, bright young people escaped from the villages in which they were brought up by joining some influential religious order and moving on a wider stage, so in the Soviet Union there is a constant and substantial flux of young bright people through the special schools and through the universities into the professions, the scientific professions among them. For other people, it also helps that the modern equivalents of the lords of the manor — the local party bosses — are susceptible to influence: a person may reasonably expect to improve the lot of himself and his family and never get into trouble provided he is willing never to ask for what may be forbidden. For every Sakharov, there may be 100,000 content to live within the system. The course of events will be determined not by the number of open rebels but by the unknown influence of those with similar inclinations still undeclared.

So does it follow that Sakharov and his fellows in adversity have no choice but to wait for a counter-revolution? That gloomy logic is fortunately unsound. Although the feudal practices of the Soviet government have the obvious advantage of keeping the government of the day in power, there is no reason to suppose that they are essential to the survival of socialism, communism, Marxism or whatever blend of those ingredients may be fashionable. To be sure, it is a depressing truth about the past half century that most communist governments have kept their ideology intact by restricting liberty, if only at the margin. The fate of Mr Michael Manley's government in last month's elections in Jamaica will no doubt reinforce them in their prejudices. Yet is it not ironical that many of those who find themselves in Sakharov's plight are as concerned with the survival of the Marxist state as are their governments? Liberty need not spell the end of the Revolution even though each year that passes makes the conflict between them sharper.

No choice

So what is to be done? The wisest assumption is that nothing much will change: that Madrid will be a disappointment but that the Helsinki accords will remain in being; that Mr Reagan's government in the new year will reopen discussions about arms limitation but will not embrace detente with much enthusiasm; that Soviet troops will stay indefinitely in Afghanistan but will not take day t day charge in Poland; and that Soviet scientists will not be free to travel overseas. Western governments at Madrid will no doubt make the best they can of what the scientific community has been asking of them in the past few months, but are likely afterwards to be preoccupied with other things. The scientific community will then be left to its own devices, which are primitive yet potentially effective. The objective must be to strengthen, and not let lapse, the recent pathetic trickle of intercourse with Soviet scientists on scientific matters in the hope that there will be a growing company of Soviet scientists prepared to acknowledge the value of liberty. It sounds an insubstantial remidey, yet the scientific enterprise is more robustly international in character than other branches of scholarship. The scientific community is now challenged to show that its faith in this principle can be upheld. And that is the best hope that Sakharov will not still be living against his will in Gor'kii for many winters to come.

Reagan's election cheering for science?

Harder line promises more spin-off funds

Washington

A big step for US politics, but a relatively small step for US science seems to be the main verdict on last week's presidential and congressional elections, with scientists unlikely to suffer as much from a swing to the right as other recipients of government spending.

President-elect Ronald Reagan has already said that he wants a 2 per cent across-the-board cut in the budgets of all federal agencies next year. But he is on record as supporting increased funds for research and development, a larger defence budget (with its spill-over into basic research) and the retention of both the Office of Science and Technology Policy (OSTP) and the post of President's Science Advisor.

The Republican takeover in the Senate has been as significant as Reagan's victory. The defeat of a significant handful of liberal Democrats will mean a major shift in the content of legislation. Again, however, the science budget may escape serious cuts, partly because key committee positions are likely to be filled by liberal Republicans, such as Senator Charles Mathias of Maryland, who have long been supporters of increased research funding.

Although little was said about science during the presidential election campaign, Mr Reagan has made it known in the past few weeks that he favours increased spending on science, particularly in areas related to defence needs. His main publicly-stated reservations were about the organization of OSTP, whose new staff would, he said, gather information in an informal way "rather than relying on 'blue ribbon panels' which are often partisan and work in secret".

The key question is who will fill the major science and technology positions in the new Reagan administration. Many of these choices are unlikely to be made until the new year. But preliminary discussions were held last weekend by a task force whose members indicate the likely directions of a Reagan science policy.

The task force was brought together by Dr Simon Ramo, co-founder of the advanced engineering firm TRW Inc., an adviser to Mr Reagan during the election campaign and former chairman of a congressionally-established federal science and technology committee under President Ford.

Co-chairman of the task force is Dr Arthur Bueche, vice-president of research and development for the General Electric

Company, a strong supporter of nuclear power and a possible Science Advisor.

Other members of the task force include Dr Harold Agnew, past director of the Los Alamos Scientific Laboratory; Dr Ed David, president of Exxon Research and, for a time, President Nixon's Science Advisor; Dr Frederick Seitz; Dr Guy Stever, ex-director of the National Science Foundation (NSF) and President Ford's Science Advisor; Dr Bill Baker, past president of Bell Laboratories; and Dr Edward Teller, a close adviser to Mr Reagan when he was governor of California.

Also included are several senior administrators from the defence and aerospace industries, including Dr Albert D. Whelan, vice-president of the Hughes

Aircraft Corporation and a strong supporter of the commercialization through private industry of communications and Earth monitoring satellites.

One immediate task for the committee is to select a new administrator for the National Aeronautics and Space Administration (NASA) to succeed Dr Robert Frosch, who has already announced his intention to take up the position of president of the new National Association of Engineering Societies in January.

Less certain is whether an immediate successor will be sought for Donald Dr Fredrickson, director of the National Institutes of Health. Dr Fredrickson was

Plusses and minuses, election 1980

Plus

Fast breeder research. One of Mr Reagan's first actions as President is likely to be to lift the embargo on the construction of the Liquid Metal Fast Breeder Reactor at Clinch River in Tennessee. Most of the major components have been built, but the test reactor's completion was held up by President Carter because of proliferation dangers and also a belief that fast breeder reactors are unlikely to be needed in the United States until well into the next century. Utility companies, however, which have provided a substantial amount of the funds, are keen to see the project completed, and are now likely to get their way.

Chemical weapons. President-elect Mr Reagan is also likely to give the go-ahead to a new facility for producing binary chemical weapons, favoured in Congress but, following President Nixon's earlier ban on their production, requiring a presidential declaration that such weapons are needed. In a campaign statement, Mr Reagan said that since binary weapons are much safer to stockpile and transport than existing stockpiles of poison gas, "we should proceed with preparation to convert to binary weapons".

Science court. In another statement, Reagan also said that he would explore the feasibility of a "science court" to help arrange public discussions of controversial scientific issues, a proposal that generated considerable publicity four years ago, but has since lapsed into relative obscurity.

House of Representatives. The House has lost one of its three scientist members, Mr Mike McCormack (see below), but has gained another, Mr Jim Coyne, an energy analyst and president of the Coyne Chemical Corporation. With Mr Coyne's election, all three scientists in the House are now Republican.

Minus

Mr Mike McCormack, previously chairman of the House Science and Technology Committee's powerful energy research subcommittee, a strong advocate of nuclear power and a leading architect of recent nuclear fusion research legislation. Mr McCormack lost a tight race in Washington, where his district included the Department of Energy's Hanford Reservation.

Mr Jerome A. Ambro. A Democrat from Long Island, Mr Ambro was chairman of the Science and Technology Committee's subcommittee on natural resources and environment. He played a significant role in generating congressional approval for the 400-GeV particle accelerator, ISABELLE, at the Brookhaven National Laboratory.

Environmental regulation. The loss of liberals such as Senators Frank Church (Idaho), John Culver (Iowa) and Gaylord Nelson (Wisconsin) and Representatives Bob Eckardt (Texas) and Andrew Maguire (New Jersey) will be a major blow to supporters of tough environmental regulation. Their only consolation is the re-election of another liberal, Congressman Morris Udall, chairman of the House Interior Committee. Mr Reagan has promised to repeal environmental and health legislation that is considered unnecessarily burdensome to industry.

Alfred Carnesale. The change in administration makes it unlikely that, as proposed by Mr Carter, Dr Alfred Carnesale, professor of public policy at Harvard's Kennedy School of Management, will now become chairman of the Nuclear Regulatory Commission. Although respected as a negotiator, Dr Carnesale is closely identified with a Ford-Mitre study that strongly influenced President Carter's stand against fast breeders.

David Dickson

initially appointed by President Ford and was retained by President Carter; he had previously indicated that, even if Carter won, he would probably leave some time next year.

At NSF, although the Senate has approved the nomination of Dr John Slaughter as the new director, he has yet to be sworn in by the President and it is possible that Mr Reagan will wish to propose another candidate for what is supposed to be a relatively non-partisan appointment.

In the Senate, much will depend on how the chairmanships of the various committees are allocated. One of the most prominent election casualties was Senator Warren Magnusen, long-time representative of the state of Washington, who as chairman of both the Senate Appropriations Committee and its health subcommittee had presided over a period of substantially increased funds for biomedical research.

If the Democrats had retained control, the subcommittee chairmanship could have passed to Mr William Proxmire, the scrouge of NSF. As ranking Republican, however, the position could now be taken by Senator Mathias — unless he prefers to take over Mr Proxmire's committee, which is responsible for the budgets of both NSF and NASA.

NASA's hopes for a relatively sympathetic hearing in the Senate have also been raised by the likelihood that the chairmanship of the Commerce Committee's science, technology and space committee will pass to Republican Senator and former astronaut Jack Schmitt, a keen supporter of the space programme and of increased funds for space science.

In the House of Representatives, which remains in the hands of the Democrats, the main changes will result from a possible reorganization of the Science and Technology Committee, where three subcommittee chairmen — Mr Mike McCormack, Mr Jerome Ambro and Mr John Lloyd — all lost their seats.

David Dickson

Polish unions

Science not lost

Gdańsk

In spite of the legal wrangles over registration and the need to prepare contingency plans for a possible nationwide protest strike, Poland's new independent self-governing trade union, *Solidarność*, has already started work. Among other things, it established, at the end of last month, its All-Poland Coordinating Commission for Science.

This at first glance is somewhat surprising, because the present policy of *Solidarność*, as the very name implies, is to build a strong union organization without subdivisions by trade or profession. Profession-based branches were originally

planned for a later stage. Such a policy meets with the aspirations of the many briefly established new unions which, after the creation of *Solidarność* in mid-September, were quick to amalgamate with it. As Dr Zdzisław Bibrowski, erstwhile chairman of the Independent Trade Union of Scientific, Technical and Educational Workers (founded 8 September — see *Nature* 18 September — merged with *Solidarność* 13 October) explained, the members felt that in the present fluid situation they needed greater bargaining power and protection than a single small union could afford.

The *Solidarność* commission for science is not intended to be a separate interest group but rather a working party to elaborate the union's practical suggestions on the strengthening and renewal of the role of science in Poland.

Such a renewal is widely accepted as a major factor in getting the Polish economy out of its present crisis. It was the theme of last month's extraordinary General Assembly of the Polish Academy of Sciences and of a number of statements by academic and scientific bodies throughout the country. Lech Walesa, the leader of

Solidarność, told me last week "Speeches and declarations are not the point. We need concrete activity."

Such activity will be the role of the *Solidarność* commission. As Dr Wojciech Gruszecki, a chemistry lecturer at the Gdańsk Technical University and the *Solidarność* spokesman on science and technology explained, the commission, which has already met several times in Poland's leading academic centres (Krakow, Warsaw, Gdańsk, Wrocław) hopes to work out some concrete proposals and submit them to the government; so too during the next few months will the Academy of Sciences and the country's various learned bodies. New legislation on science in Poland is now being drafted and will shortly be presented to the Sejm (parliament). In the current atmosphere of greater "openness" a lively public debate is expected.

On many points, including the need for greater autonomy in academic life and the lifting of censorship restrictions, the scientific and academic community is unanimous. *Solidarność*, however, would go further. As Dr Gruszecki explained, the union urges that science be treated as a non-

University of Warsaw changes course

Warsaw

The expected liberalization of Polish academic life is not entirely a consequence of the Gdańsk accords, according to a spokesman for the Polish Ministry of Higher Education and Science. Rather, he said, it is the cumulation of a continuing process. Thus the promise of a greater degree of self-government for the universities was already foreseen in guidelines drawn up several months ago.

The ministry, it appears, wishes to seem well abreast of developments. This has been especially the case since the surprise resignation at the end of September of the rector of Warsaw University. With widespread talk of academic autonomy, the ministry found it prudent to offer a compromise solution for the appointment of his successor. It proposed a list of three candidates, from whom the university senate made its final selection.

The senate is now committed to the principle of academic autonomy. The pro-rector, Dr Kazimierz Dobrowolski, stresses that the university should be able to draw up its own syllabuses and courses. Even under the present system, he says, some latitude is possible. In his own subject, biology, the university has been able to initiate courses in such new fields as environmental biology and molecular genetics. The result, he says, is that no student can graduate with simply a nineteenth century knowledge of biology.

The academic staff at the university is

nevertheless concerned with falling standards among candidates for entry. The educational reform, introduced three years ago, which replaced the traditional eight years of primary schooling and four years lyceum by a comprehensive ten-year schooling, alarmed both parents and university staff. Although three years is strictly too short a time in which to assess the new system, the Ministry of Education has been quick to read the signs. Although no official announcement has yet been made, a recent article in the weekly *Perspektywy* suggests that the new Minister of Education, Krzysztof Kruszewski, intends to revert to the former system, with certain changes in syllabus incorporated from the ill-fated ten-year system.

A recent development at Warsaw University has been the inauguration of a course of Friday evening lectures by members of the Society for Academic Courses (the "Flying University") whose aim is to fill the gaps left by the state system of instruction. The lectures, held in the Adam Mickiewicz Hall (the largest lecture room in the university) are not, however, formally sponsored by the Society for Academic Courses. The hall is leased to the new Independent Students' Union, which invited the speakers. But, as one of the student organizers said, the correspondence between the list of lecturers in the Mickiewicz Hall and the members of the Society for Academic Courses is hardly coincidental.

Vera Rich

commercial activity and financed by a budget which would ensure the total autonomy and freedom of scientific research. Research, he said, should not be "cramped" by government plans. Industrial research should be carried out in the research institutes of the ministries concerned (a network of such institutes does exist), or contracted out to academic institutes; but the overall direction of research by the government should cease.

In effect, Solidarność is seeking a major change in Poland's science policy. Since the early 1970s, research has been organized and funded on the basis of a complex hierarchy of "problems" (governmental, key, interdepartmental, departmental and branch problems) geared to the needs of the national economy. Research on a given problem will generally be carried out at many institutes simultaneously, and problems of coordination and control, including the interface between the academic and governmental sides, have led to a proliferation of bureaucracy. The establishment view, however, as represented by Dr Ignacy Malecki, director of the Institute of Fundamental Problems of Technology of the Polish Academy of Sciences, is that the problem structure is not in itself a bad thing. Dr Malecki, a member of a special commission looking into the management of the "problem" system, said that the consensus was that the system should stay, although trimmed of its bureaucracy. He added that in his opinion there was room for improvement in financing. **Vera Rich**

Science council

Mess about money

The UK Science Research Council is in a financial mess. But nobody can say how serious the trouble is, because figures and tables released in the 1979-80 annual report last week are wrong. The SRC now confirms the misuse of a computer program, as a result of which some research grants which have ceased have been displayed as current. A claim in the report that grant payments had increased by 72 per cent since the previous year took SRC officials four working days to disown once the inconsistencies had been pointed out to them.

Behind this is a more serious problem — what chairman Sir Geoffrey Allen last week called a "hiccup" in the council's cash flow. He and his officials are being coy about the size of this financial spasm. Some say that the council must find at least £10 million from within its current budget to pay for excesses last year in grant-giving by the Science Board, responsible for small science and neutron and synchrotron radiation physics, thus shedding doubt on claims earlier this year that the Science Board was turning away "alpha quality" grant applications for lack of cash.

The anomaly dates back to a success:

Hardship everywhere

The Science Research Council's report for 1979-80 (HMSO, £7.10) is liberally spattered with references to the consequences of financial stringency, within a budget totalling £176 million. The budget last decreased 1 per cent in real terms in 1980-81, and will show a modest increase after that — but, says the report, new initiatives will be impossible without producing stagnation in favoured areas of the council's programme, or markedly reducing other programmes.

Nuclear physics is the most vulnerable of the big spenders — although contributions to CERN fell from £27.7 million in 1978-79 to £25.3 million in 1979-80 largely because of the stronger pound. The nuclear physics budget is not one of the "favoured areas" of the SRC's programme, and it has fallen a factor of two in real terms since 1975-76. It will fall again by 18 per cent this year, and another 9 per cent the year after, says the report, to £2 million below a "minimum programme"; but despite this the board sees LEP, the large electron positron ring that CERN plan, as its accelerator for the late 1980s, as its highest priority. It is "seeking council support" on this project. Meanwhile, equipment for the 30 MV Van de Graaff Nuclear Structure Facility under construction at Daresbury is to be further delayed.

X-rays and neutrons provided by the synchrotron radiation source at Daresbury, opened last week, and the Spallation Neutron Source under construction at the Rutherford Laboratory are threatened by lack of money to exploit them; and existing neutron sources will be run down. The high proportion of the Science Board's funds devoted to neutron scattering will fall after 1981-82, when the modernization programme at the Institut Laue-Langevin high flux reactor at Grenoble, in which the UK is a partner, is completed.

Computers. The SRC needs to find £8 million capital "over the next few years" to replace its central computers, and more if allowance is to be made for an increasing demand.

Research students supported by the SRC will continue to be a falling proportion of good graduates in science or engineering. In 1972, 46 per cent of such graduates, applying for SRC grants, were awarded them. In 1979-80 the proportion was 36 per cent, and now the number of grants must be held constant against an increase — for the next few years — of about 4 per cent a year in university output. But there should be a few more cooperative awards in science and education (CASE awards), designed to link students with industry as well as university. Even so, this council hopes to fund more in-service training.

Robert Walgate

that of Mrs Shirley Williams, Secretary of State for Education and Science in the last government, in convincing her Cabinet colleagues that Britain should spend more on science. Uniquely, she brought in research council heads to convince other ministers that basic science was important — and in late 1978 won another £47 million for the councils, of which SRC would have received £33 million, payable from 1979-80 to 1982-83. Then came the new government; spending cuts, announced in the budget of June 1979, lopped off most of Mrs Williams's gift.

But well before the election she had privately warned the council chairmen to beware of Tory zeal. In case the government changed, she hinted, it would be wise to spend the money soon. In April 1979, SRC sent out a circular requesting applications for grants to buy equipment. £37 million of applications flooded in; SRC panels thought £20 million of them were deserving, and committed themselves to £7.5 million. In the event, the council received only £5 million of the Williams money.

The flood of applications, however, continued, and not just for equipment. The universities had seen the tap turned on after years of drought. It was against this background that "alpha quality" applications had to be turned down. Even so, the boards over-committed themselves and now all are being asked to cut back by delaying grant renewals.

Other factors worsen the SRC's current financial picture. Universities are putting in their bills earlier than usual, because they too have cash flow problems; and the largest price of SRC construction work — the spallation neutron source at the Rutherford laboratory — is going ahead on time (the council had allowed for slippage).

The only light comes from the strong pound, which reduced the council's subscriptions — totalling £42 million a year — to international bodies such as the European centre for subnuclear physics (CERN) and the European Space Agency by about £7 million; and from the volcanic rock underneath the new Isaac Newton telescope house at La Palma in the Canaries. Engineers had overestimated the strength of the rock, and the foundations must be rebuilt, causing a six-month delay and pushing a £500,000 bill into next year.

Robert Walgate

Large space telescope

Delay means money

Washington

US astronomers have successfully persuaded the National Aeronautics and Space Administration (NASA) to request additional funds in next year's budget to ensure the successful development of scientific instruments due to fly on the space telescope.

Previously, scientists had complained

that significant cost and schedule overruns with the 2.4-metre telescope, whose planned 1984 launch from the space shuttle will probably have to be postponed for up to a year, were leading the agency to cut corners in developing and testing the instrumentation thus prejudicing some of the scientific experiments.

For example, no reserve funds to meet unexpected difficulties had been included in the budget of the Goddard Space Flight Center, which is responsible for the development of the scientific instruments which will be located at the telescope's focal plane.

Partly as a result of this delay, caused chiefly by greater than expected technical difficulties in various parts of the project, cost overruns could amount to \$50–\$100 million, compared with an initial estimate of \$435–\$470 million.

The space telescope is only one of a growing list of NASA projects in the same trouble. In some cases, such as the Galileo mission to Jupiter, and the solar polar mission — delayed two years — these have been the direct result of problems with the space shuttle itself.

Ariane sells three

Washington

Intelsat — the international telecommunications satellite organization — has given a major boost to European space efforts with its decision to launch three of its nine satellite global communication systems from the French-built Ariane rocket.

Intelsat had originally hoped to launch the satellites from the National Aeronautics and Space Administration (NASA)'s space shuttle. Delays in the shuttle development programme, however, have caused concern about whether the launcher will be available in time. (Even after the first launch next year, it is uncertain whether NASA will be able to keep up its promised schedule of launches.)

Ariane is also experiencing problems, and following the failure of a test flight earlier this year there is no guarantee that it will be available for the Intelsat launches, which would be the sixth, seventh and eighth in the nine-satellite, \$300 million system. In this case, Intelsat would use Atlas Centaur rockets, which because of the shuttle delays are already being used for the first five satellite launches, with the first launch due to take place next month.

The launch vehicle for the final satellite in the series has not yet been decided, because Centaur launches are likely to be considerably more expensive than the shuttle. When all satellites are in orbit, the system will double the number of international telephone calls and television transmissions that can be handled simultaneously.

David Dickson

But in the case of the space telescope, there have been separate problems with each of the three parts of the project: the scientific instrumentation, the optical telescope assembly and the support system module.

In the case of the scientific instrumentation there have been what one scientist describes as a not unusual set of technical problems, extra costs caused by delays and a few "minor screw-ups" in all instruments except the high-speed photometer.

It is a similar story with Perkin-Elmer, which was awarded a \$58.5 million contract for the optical telescope assembly and which has also had difficulty in keeping work on schedule, finding that it lacked sufficient manpower for design needs and that a computer-controlled method for grinding one of the two optical mirrors was not as successful as had been hoped.

More recently, the other main contractor, Lockheed, awarded a \$73 million contract for the support systems module, reported that revised cost estimates were substantially larger than those originally submitted.

Both NASA officials and staff members of the relevant congressional oversight committees are looking closely for the reasons behind the delays. In particular, Congress wants to know whether tight limits on personnel have reduced the agency's ability to review contracts awarded to private companies.

In Europe, where several companies are working on apparatus to be included on the telescope, a delayed launch will relieve pressure on schedules that were said to be getting tight.

The delay seems unlikely, however, to have a significant impact on plans for a space telescope institute, which will be responsible for analysing the data received from the telescope.

NASA is expected to announce within the next few weeks the site that it has selected for the institute. Of the original four proposals submitted earlier this year, two remain in the running. One is from the Association of Universities for Research in Astronomy (AURA), which proposes building the institute at Johns Hopkins University in Baltimore; the other is a proposal from Associated Universities Inc. to locate the institute at Princeton University.

Although both proposals are accepted by astronomers as having strong merits, the balance may have been swung in favour of Princeton by a recent federal indictment against Computer Sciences Corporation (CSC). This company, which AURA intended to use to help develop the software for handling telescope data, is being indicted on charge of fraud and racketeering arising from a 1972 time-sharing contract.

AURA officials pointed out last week, however, that the section of CSC which has

been indicted is completely separate from that which would help operate their proposed space telescope institute, and hope that this will insulate them from any action which the federal government might take against the company. The General Services Administration (GSA), against which the frauds are said to have been carried out, has suspended the company from acquiring new business from the agency until a trial which is expected to open in January. The action by the GSA could affect 200 federal users of CSC's Infonet time-sharing system.

David Dickson

Biotechnology

Celltech set up

The formation of Celltech Limited, the first British company predominantly concerned with the exploitation of biotechnology, was completed on 6 November, according to an announcement earlier this week from the National Enterprise Board (NEB), the public corporation supported with public funds. The board will be the largest shareholder in the company, with 44 per cent of the shares. The remainder of the equity will be divided equally between the four remaining shareholders, the Prudential Assurance Company, the Midland Bank, British and Commonwealth Shipping and TDC (a venture capital investment trust, itself an offshoot of Finance for Industry, a joint venture between the clearing banks and the British government).

The shareholders have already subscribed the first tranche of the £12 million that will serve as the initial capital base of the company. The board of the company will chiefly be representative of the principal shareholders, with Mr G. Jackson of Philips Electronics Limited nominated by the National Enterprise Board (NEB), but will also include Dr J. L. Gowans, the secretary of the Medical Research Council, and Sir Michael Stoker, until recently director of the Imperial Cancer Research Fund Laboratories and foreign secretary of the Royal Society. Sir Michael will also be the chairman of the company's advisory board, to be known as the Science Council.

The council will also have as a member Dr Sydney Brenner, director of the MRC Laboratory for Molecular Biology at Cambridge, who has been involved with the planning of Celltech from the beginning. Other members of the council are to be announced in the near future.

Apart from the managing director of the company, Mr S. Fairtlough, the director of research and development will be Dr N. Carey, at present director of research at G. D. Searle Limited. Dr Caroline Vaughn, like Mr Fairtlough at present on the staff of the board, will be director of marketing. Other members of the staff of the company have yet to be appointed. The chairman of the company will be Mr Wynn Denman, a

director of British and Commonwealth Shipping.

The announcement of the setting up of the new company said that Celltech has reached an agreement with the Medical Research Council (MRC) that provides a "framework" within which the company will have access to research carried out in the council's laboratories. It has also come to an arrangement with the National Research Development Corporation, the public corporation which is the principal repository of patent rights arising from research carried out in university and public laboratories in the United Kingdom. Plainly the negotiation of these agreements, no details of which have been made public, has taken up some of the time that has elapsed since the Celltech project was first announced nearly four months ago (see *Nature* 24 July).

Veal hormones

Total ban proposed

Brussels

The Commission of the European Economic (EEC) has lost no time in taking steps to ban the use of all natural and artificial hormones in livestock — a draft regulation is due to come into effect on 1 January 1981. At the Council of EEC Agricultural Ministers on 30 September the Commission promised to respond speedily to a request to draw up proposals on the use of hormones and anabolic steroids. This week's Agricultural Council meeting on 10 and 11 November was to be given an account of the Commission's proposals, but will hardly have had a chance to digest them.

Although the ministers have committed themselves to speedy action there are several hurdles ahead of them. The proposals are far reaching — covering all meats intended for human consumption (veal, pork, beef and fowl). The only exception is for the use of natural hormones in therapeutic treatment, which must be carried out under strict supervision. Furthermore, the marketing of animals or carcasses containing residues above a certain level (yet to be specified) will also be prohibited.

None of this will be easy to enforce. The commission still has to devise a foolproof way of controlling the supply of hormones to vets, ways of monitoring animals from the farm to the abattoir, and ways of checking meat on retail sale. These controls are bound to involve a system of identification so that the animal's origin can be traced if the regulations are broken.

The commission's proposals will be particularly welcome in Italy, where in September a magistrate imposed a nationwide ban on veal sales and the sale of twenty-two brands of baby foods containing veal was suspended. The British position is one of traditional restraint, partly due to unwillingness to yield too

much power to Brussels and partly due to scepticism over the implementation of EEC regulations. This scepticism is shared in Germany, where laws on the import of meats have already been tightened.

Jasper Becker

Medical research

Born-again basics

The UK Medical Research Council (MRC) and the departments of health in London and Edinburgh are making a valiant effort to defend the transfer of Rothschild contract research monies back to the control of the MRC (*Nature* 23 October, p.669). Last week, on the publication of the MRC annual report for 1979-80, MRC secretary Dr J.L. Gowans said that his council is expected to double its commitment to "health services research" in the next five years. And Professor Arthur Buller, Chief Scientist at the Department of Health and Social Security, said that his department had pressed the MRC into the deal, rather than the other way about.

The doubling of health services research is said to be the MRC price for gaining control of the £13.85 million per annum of Rothschild cash. Since 1972 and the adoption of the Rothschild customer-contractor principle, these funds have been controlled by the health departments but spent by MRC. Under a concordat reached in 1973 between the departments and the MRC they were spent exclusively on biomedical research. Each year the departments defined their interests in a voluminous report under headings such as "blood", "mental illness", "cancer and infections" and so on; and MRC responded, detailing exactly how its biomedical research was relevant. Each year, the health departments passed the Rothschild allocation to MRC, duly increased for inflation.

Health services research, which involves social and economic as well as medical matters (for example, does amniocentesis yield a net benefit?) was, strangely, not covered by the 1973 concordat — so that the £2 million or so a year of health services research at present undertaken by the MRC was in fact funded with money outside the Rothschild principle.

The health departments themselves have thus pressed MRC into the new arrangement under which MRC must spend an extra £2 million on health services research by 1985. Professor Buller, no fan of the 1973 arrangement, considers this a net gain for health services researchers, who might expect, for example, two or three new MRC units in the area by 1985.

Both Professor Buller and Dr Gowans, however, insist that the definition of health services research must be broad, and that the creation of new units will depend on the availability of good people to head them. The agreement is to be reviewed in five years, when the health departments could

MRC report in brief

The 1979-80 report makes the following specific points:

Neutron therapy. Another £1½ million is needed by the end of this year to buy and set up a new cyclotron for neutron therapy research at Clatterbridge Hospital, Liverpool — which would thus overtake the Hammersmith Hospital, London, in this work. The cyclotron would be outside the MRC budget, and would be funded largely by the Imperial Cancer Research Fund, the Mersey Regional Hospitals Authority, and a Liverpool cancer research fund. But quotations for the equipment expire in December, when inflation would probably put the cyclotron out of reach.

Tropical medicine. The Tropical Medicine Research Board of MRC met last Friday for the first time since 1979, having cancelled two meetings for lack of money for research grants since cutbacks in Overseas Development Administration spending in 1979. No new projects could be funded — and overseas cooperation is threatened by the new round of government spending cuts. Recruitment into the field has halted.

Budgets. Total expenditure by MRC in 1979-80 was £74 million, including £57 million from the Department of Education and Science and £12.7 million of Rothschild money. The council notes with approval the stability promised in the most recent public expenditure plans, but is apprehensive about the effects of cash limits in inflationary times. Anxious to increase its room for manoeuvre, the council says it has deliberately reduced its commitments to long-term research programmes. During the year, it opened two new research units and closed seven.

Private practice. The council has confirmed its longstanding policy that members of the MRC research staff should not be allowed to engage in private medical practice. The review was made necessary by the decision that physicians working for the National Health Service might take on private patients. The MRC council says that private practice would be incompatible with full-time research appointments, promises to secure adequate salaries for those members of its staff concerned and hopes that British universities will follow a similar policy.

Robert Walgate

claw back the money if MRC has not performed according to plan. MRC should undertake "brick upon brick" health services research, says Buller, without the constraint of contracts tied to particular policy objectives. The subject is just at the point of requiring such academic treatment, he believes, and it is best handled under the dual support system. The departments' own £20 million of research money will continue to be spent on pragmatic matters.

Robert Walgate

CORRESPONDENCE

Sakharov

SIR—Your journal of 11 September contained a short article by Vera Rich about a conference in the Hague devoted to the "Sakharov affair". On the basis of this article (and I have no grounds for doubting its accuracy, so my grievances are not directed at the author and editors) I was left with the impression that the participants of the conference had received distorted information. This information then reached the pages of your journal. Factual inaccuracies in the description of my situation at the conference and other significant facts and a kind of general demobilizing trend of these inaccuracies aroused perplexity and anxiety, causing me to send this letter to your journal.

I suppose that the participants of the conference and its organizers sincerely wished to help me and others who are suffering from repression, but excessive haste and other errors in its organization have almost cancelled its usefulness.

In my time I have taken part in defending the organizer of the conference, Dr Shtern, but I have never made his acquaintance. I have had no communication with him during the preparation for the conference or at any other time. I am also not acquainted with Dr Lozansky and did not meet him at the Jewish seminar of "refusenik" scientists in Moscow. Shortly before the start of the conference Dr Shtern contacted my representative abroad, Efrem Yankielevich, with a request for him to participate in the work of the conference. Yankielevich declined, expressing some apprehension concerning his unpreparedness, though he submitted some concrete advice about desirable witnesses (which, as far as I know, was not used). Yankielevich also commented on the excessively pretentious (in his, and my, opinion) title of the conference.

The article, however, does not refer to Efrem Yankielevich but to his wife, my adopted daughter Tanya Yankielevich (erroneously called Tamara), who it seems objected to "garish" actions, as if they might interfere with the plans of the Soviet authorities to return me to Moscow. This is not simply an inaccuracy, but something outrageous. A stance that is completely alien to us is ascribed to a person close to me, and thereby, indirectly, to myself also.

I, and persons of a kindred spirit, regard openness as the only weapon in the fight for justice. The plans of the authorities are known to no-one. Our actions and statements must be based on fundamental considerations as we perceive them, without accommodating ourselves to these plans, which are unknown to us, or to anyone's ambitions or other considerations of the political situation. This applies both to my defence, and to the defence of other victims of repression, and to general questions such as disarmament and international security, human rights, environmental protection and nuclear power.

Now to some concrete inaccuracies. In Moscow, publication of preprints of my articles in English was forbidden, and the manuscripts were removed by the KGB from the office of the Institute, evidently to preclude any possibility of acquaintance with my work in the West. The preprints were published at Stanford University, and I am deeply grateful to my colleagues there. I am also very grateful to my colleagues in the USA and in other countries who send me their preprints. The Russian texts of my articles are published in *Zh. éksp. teor.*

Fiz. in June, August and September, according to normal schedules. I am confident that the Politbureau has had nothing to do with these publications, and, on the whole, references to the Politbureau without stating the sources of information seem unfounded.

My isolation in Gor'kii is of an entirely different nature than appears from the conference. There is no scientific library opposite my home, to which I might have access. Opposite there is only mud and piles of rubbish. I have no contact with scientists in Gor'kii, not because there are only secret institutes here, but because I am in a state of almost total isolation, deprived of the possibility of meeting anybody at all apart from my wife and two people from Gor'kii, who obtained permission for this from the KGB, and one visit from my university colleague, also by permission of the KGB. Any others are kept away by a militiaman, on duty round the clock, one metre from the door.

I don't even get to know about the majority of visitors, and they have great trouble. After some time I merely learn of people who are close to me. Our friend and doctor, who travelled from Leningrad, was not admitted, nor was our 82-year old aunt from Moscow. They do not even admit my son's fiancée, who has lived with us nearly three years — Liza Alekseeva. The authorities will not allow her out of the country to join the person she loves, she is subjected to persecution, threats of physical and legal reprisals. Fear for her, for her life compels my wife to divide her time between Gor'kii and Moscow. Liza Alekseeva has become a hostage of my public activity, and now her defence is, perhaps, the most realistic form of supporting me.

To describe my situation, I might add that I have no telephone and it is not possible to make a call from a post office; I am deprived of the medical aid of those doctors who used to treat me; my correspondence is carefully inspected by the KGB and only a fraction of correspondence reaches me; in the house where I live there is a personal radio jamming device, which was even in operation before jamming of radio transmissions was resumed in the USSR.

In July my wife found two KGB agents in the flat, who had entered through a window and, without the knowledge of the militiaman on duty, rummaged through my papers, and erased tape recordings. Illegal entry like this, the purposes of which may be even more dangerous, has happened before. I have not received a reply to a single one of my letters or telegrams to officials. Two months ago I sent a letter to the vice president of the Academy of Sciences of the USSR, E. P. Velikhov, and would like to hope for a reply.

The Soviet press, Soviet representatives abroad and some of my Soviet colleagues during foreign missions, in contacts with people in the West who are concerned about my fate, in an attempt to disorganize my defence, assert that I am against détente, have spoken out against SALT, and have even permitted the divulgence of state secrets; they also emphasize the mildness of the measures taken against me. My attitude and open way of life and actions are well known and show how absurd these accusations are.

I have never infringed state secrecy, and any talk of this is slander. I regard thermonuclear war as the main danger threatening mankind, and consider that the problem of preventing it takes priority over other international problems; I am in favour of disarmament and a strategic balance, I support the SALT-II agreement as a necessary stage in disarmament

negotiations. I am against any expansion, against Soviet intervention in Afghanistan, but in favour of aid to refugees and the starving throughout the world. I regard as very important an international agreement on refusal to be the first to use nuclear weapons, concluded on the basis of a strategic balance in the field of conventional weapons. I am convinced of the interrelatedness of international security and defence of human rights, and am in favour of freedom of convictions and exchange of information, freedom to choose one's country of domicile and place of domicile within that country, and freedom of religion. I am deeply anxious about the fate of political prisoners in the USSR, unjust courts, illegal repression. The most important aim for me is the release of prisoners of conscience throughout the world, including the USSR, the countries of Eastern Europe, and China.

I do not make it my task to give special support to the viewpoint of Western governments, or anyone else, but express precisely my own viewpoint on matters causing me anxiety. As for the mildness of the measures taken against me, they are not as severe as the terms of imprisonment lasting many years for my friends and scientists—prisoners of conscience Sergei Kovalev, Yurii Orlov, Toli Shcharanskii (Anatoli Shcharansky), Tanya Velikanova, Viktor Nekipelov, nor as the fate of those awaiting trial — Aleksandr Lavut, Leonard Ternovskii, Tanya Osipova and many others.

But my banishment, without trial in infringement of all constitutional guarantees, the isolation measures applied, interference of the KGB in my life, are completely illegal and inadmissible as an infringement of my personal rights, and as a dangerous precedent of the actions of the authorities, who are casting aside even that pitiful imitation of legality in the persecution of dissidents that they displayed in recent years. Only a court has the right to establish that a law has been infringed and to define the manner of punishment. Any deliberations about culpability and mercy without a trial are inadmissible and against a person's rights.

Therefore I insist on a public trial, and attach fundamental importance to this. It is this that should have been heard at the conference in my defence, and not the greater or lesser mildness of the measures taken against me. Unfortunately, albeit unwittingly, the conference adopted the formulation of the problem that is most advantageous to the Soviet authorities, but even then there were the factual inaccuracies stated above.

I do not know how the fate of others was described (this is not mentioned in the article), but the report concerning the only other person apart from myself mentioned in the article — Dr Gol'fand — is incorrect. He was not dismissed from his work in connection with an application to leave for Israel, but long before he decided to emigrate. Those colleagues of his in the West who are helping him in his struggle for the right to emigrate will, I fear, be misinformed and will cease their efforts after reading such a cheerful paragraph about his reinstatement.

In conclusion I should like to draw attention to the possibility of obtaining accurate information on my situation and attitude directly from my statements and from the reports originating from my wife and from my representative abroad, Efrem Yankielevich.

ANDREI SAKHAROV

6 October 1980,
Gor'kii 137, Gagarina 214 kv.3, USSR

NEWS AND VIEWS

Why is magnetite not a good conductor?

MAGNETITE is a remarkable material, as the first users of lodestones were well aware. When reasonably pure, it looks and feels like a metal. Lumps of magnetite such as can be picked up in some iron mines even sound metallic when struck or thrown to the ground. It is therefore worth recalling that there are some grounds for thinking that magnetite should also conduct electricity as if it were a conventional material. The argument, which of course is false, goes something like this. The crystal structure of magnetite is technically that of the inverse spinel structure. Iron cations turn up on two different kinds of sites within the lattice, conventionally known as A and B sites. The A sites are occupied by Fe^{3+} cations and are tetrahedrally related to surrounding oxygen anions. The B sites, on the other hand, have octahedral symmetry and are occupied by equal numbers of Fe^{2+} and Fe^{3+} ions. This is entirely consistent with the old school textbook notion that magnetite, Fe_3O_4 , is a molecular compound of the divalent and trivalent oxides of iron, FeO and Fe_2O_3 . Considered in isolation, however, the lattice of B sites is potentially a candidate for conduction as if it were a metal. It is possible to think of it as a lattice of Fe^{3+} cations with one conduction electron for each pair of cations. How good a conductor of electricity magnetite should be at, say, ordinary temperatures would nevertheless be a matter for detailed calculation and even speculation but one thing would seem to stand out — at some sufficiently low temperature, and certainly near the absolute zero, magnetite should conduct electricity. The fact is that it does not.

If anything, the truth is the opposite. At low temperatures, magnetite has the electrical conductivity of an insulator, but at about 110K there is a first-order transition (recognizable as a specific heat anomaly, for example) and the electrical conductivity of magnetite increases by a factor of about 100 to $100\ \Omega^{-1}\text{cm}^{-1}$. With further increase of temperature, the conductivity increases by a further order of magnitude until, as with all conductors, the disruptive effects of lattice vibrations eventually take over and the conductivity decreases with increasing temperature. The conductivity of magnetite above 110K is thus more or less what would be expected. The puzzle is to know why the material does not conduct at lower temperatures. The explanation is by no means novel, although it retains something of the flavour of a surprise. It appears that at low temperatures magnetite is not a conductor because, below the transition temperature, the electrons from the cations at B sites arrange themselves in such a way that there is some form of long-range ordering of the Fe^{2+} and Fe^{3+} ions. So much was guessed at in the 1940s by E. J. W. Verwey, after whom are now named transitions from states in which ionic charges are ordered to states in which they are not. It is salutary, therefore, to be reminded that people are still unsure what happens to magnetite below 110K. So much at least is clear from the issue of the *Philosophical Magazine* (Series B, Volume 42) for September 1980.

Magnetite is by no means the only material now known to exhibit transitions of this kind. The oxide of titanium with the composition Ti_4O_7 is another. The low-temperature state in which electrical conduction is inhibited consists of an ordered arrangement of Ti^{3+} and Ti^{4+} cations. But there are also materials typified by the samarium sulphide Sm_2S_3 in which cations of different charge (in this case Sm^{2+} and Sm^{3+}) sit on the same lattice and appear not to form a highly ordered (and thus a markedly non-conducting) state at low temperatures. Why should there be these differences? And why, in any case, is it turning out to be so difficult to come to grips with properties of

what might at first sight seem to be among the simplest of phase transitions? The special issue of *Philosophical Magazine* consisting of papers stimulated by a symposium on the Verwey transition a year ago at the Cavendish Laboratory, and obviously fostered by Sir Nevill Mott, for long the editor of the journal and who contributes an introductory paper, is a splendid way of finding out.

The first surprise is that, until very recently, the actual arrangement of the cations in magnetite at low temperatures was in doubt. That this should have been the case is no surprise if the ordered structure now described by S. Iida (*Phil. Mag.* B 42, 239; 1980) is anything like correct. Especially in the past few years, data bearing on the structure of magnetite at low temperatures have been derived from a variety of sources — Mossbauer spectroscopy, nuclear magnetic resonance measurements and neutron diffraction. The underlying difficulty is that any state in which there is long-range order of cations of different electrical charges on the B sites of the lattice is bound to entail substantial distortion of the shape of the lattice. (Some lattice distances are indeed changed by more than two per cent.) Iida's solution to what is plainly a problem of great complexity involves the description of a superlattice in which eight unit cells in the inverse spinel structure become a single unit cell in a lattice with a larger repeating unit. In the ordered state, below the first-order transition at 110K, Fe^{2+} and Fe^{3+} cations turn up as pairs or as groups of three similar cations in a row. Although the outcome of the tortuous chain of inference leading to this conclusion is outwardly simple, it is easy enough to see why it could not have been predicted, and why even now its calculation would be a formidable undertaking.

The complications are indeed even more serious than might be thought. Magnetite is not a simple conductor (above the Verwey transition) or a simple insulator (below). The interaction between the relatively large magnetic moments of the cations on the A and B sites makes magnetite a ferromagnetic material below the relatively high Curie temperature of 850K or thereabouts. (The large influence of small quantities of impurities makes the temperature uncertain.) The result is still greater strain of the lattice structure. But magnetite is also a ferroelectric at low temperatures, making for even more lattice distortion. Against this background, the recognition in recent years of further low-temperature transitions (recognizable as specific heat anomalies) is to be expected. The experimental difficulty is that the transition temperatures are sensitive not merely to the stoichiometric purity of the magnetite but to its physical condition. Iida's paper points to an explanation of the transition at 12 K, but there remains room for further argument.

The properties of Ti_4O_7 are similarly complex, although C. Schlenker and M. Marezio from the CNRS in Grenoble have a somewhat simpler tale to tell (*Phil. Mag.* B 42, 453; 1980). Two distinct first-order transitions are recognized and, at the lowest temperatures, pairs of titanium cations of different charge are frozen into the lattice. Between the two transition temperatures, pairs can migrate by a process described as 'hopping' and then, at high temperatures, long-range order disappears and the conduction becomes possible. The explicit calculations of what may be happening purport to be little more than crude approximations. Plainly, the experimentalists will make the running for some time to come. Indeed, the study of this curious and still somewhat unexpected transition is likely to serve chiefly as a pointer to fresh problems in lattice structure. □

Molecular genetics of the mouse

from Grahame Bulfield

WORKERS from two rather different areas came together at a recent conference* in an attempt to relate structure to function in the regulation of mammalian gene expression. One group of workers has been studying the large number of structural and regulatory gene loci controlling enzyme levels that have been discovered in the mouse. A second group, exploiting recent advances in molecular biology, has been looking at the arrangement and sequence of some mammalian structural genes.

The system most completely characterized by genetic analysis in the mouse is that of β -glucuronidase (K. Paigen, Buffalo; see also *A. Rev. Genet.* 13, 417; 1980) and by molecular techniques is the globin system (H. Dahl, National Institute of Medical Research, London). However, the only gene family where both approaches have already converged is the amylase one. Genetic analysis shows that the genes for pancreatic and salivary amylase isoenzymes are tightly linked. The different pancreatic isoenzymes are coded for by up to 20 tandemly repeated structural genes (T. Nielsen, Aarhus) but the sole salivary isoenzyme is the product of a single locus (J.P. Hjorth, Aarhus). These workers have identified over 20 different haplotypes of the complex in wild-caught mice and backcrossed them into the C3H inbred strain. Some of the genetically distinct pancreatic isoenzymes have been characterized by peptide analysis and partial amino acid sequencing (M. Meisler, Ann. Arbor). The A/J inbred strain has one salivary and one pancreatic isoenzyme and a single mRNA species is present in each tissue. The active salivary and pancreatic genes are respectively about 28 and 13 kilobases long and contain at least ten exons. Overall there is a family of about eight amylase-like genes, including one salivary gene, but six of the genes are not expressed in the A/J strain (R. Young, Lausanne) demonstrating a remarkable fit with the genetic evidence. The rat amylase gene has also been cloned as part of an analysis of the structure of the pancreas-specific genes and 12 amylase-like genes have been found (W. Rutter, University of California, San Francisco). The complimentary approaches of the geneticists (in providing a large number of congenic haplotypes) and of the molecular biologists (in providing methods for analysing gene structure) should produce a much better understanding of the regulation of expression of the amylase system.

The workshop provided many reports of new variation available to geneticists, the

characterization of some of the systems and their use in analysing both the regulation of gene activity and of development. Expression of β -glucuronidase is affected by several regulatory loci which map at or near the structural gene and control systemic levels of the enzyme, androgen inducibility and developmental profile (K. Paigen; K. Pfister, Zürich). The different haplotypes of this gene complex should provide valuable analytical material once the gene has been cloned.

Genetic variation in regulatory or tissue-specific mechanisms was reported for many enzyme systems including: alcohol-metabolizing enzymes (R. Holmes, Brisbane), H-2 and related systems (M. Boubelik, Prague; K. Fisher Lindahl, Basel), glycerophosphate dehydrogenase (L. Kozak, Bar Harbor), histidine decarboxylase (S.A.M. Martin, Leicester University), metallothioneins (D.M. Hunt, Queen Mary's College, London), neuraminidase (J. Peters, Harwell) and pyruvate kinase (K.J. Moore, Leicester University). Novel methods of investigating genetic regulation were introduced for teratocarcinomas (W.F. Dove, Madison), by the use of spontaneously occurring trisomics (A. Gropp, Lübeck; R. Fündele, München) and by the flow sorting of mammalian chromatin (L. Bolund, Aarhus). Many of the variants in the enzyme systems listed above have unusual features suggesting that the complexities of genetic regulation will be various and may be difficult to unravel. Further extensive genetic variation, so far little used, was shown to be available in different species (interfertile with laboratory mice) and sub-species of feral mice throughout Eurasia (L. Thaler,

Montpelier; E. Capanna and A. Gropp, Rome/Lübeck; K. Moriwaki, Mishima).

The use of biochemical variants to analyse the nature of one developmental problem — X-chromosome inactivation — was discussed in depth. An electrophoretic variant of G6PDH found in *Mus caroli* has been used to show that, in the germ cells of heterozygous fetuses, one X chromosome is active at the 10th day *post coitum*, whereas both are active between days 11 and 14 *post coitum* during the leptotene to pachytene stage of meiosis (V. Chapman, Buffalo). A 'Catherine-wheel' model has been proposed to incorporate the X-chromosome activation-inactivation cycle in the germ cells with the progressive X-inactivation of extraembryonic and embryonic tissues during fetal development (as demonstrated by gene dosage of X-linked enzymes and X-linked electrophoretic alloenzymes (M. Monk, M. Harper and A. McMahon, University College London). That X-inactivation may itself be under genetic control was shown by three alleles of the *Xce* locus which modify the extent of inactivation on the X chromosome they inhabit (B. Cattanaach, Harwell).

Most of the discussions that took place during the conference were concerned with the validity of the genetical and molecular approaches and how they might compliment one other. Two important points were made: first, the need to concentrate on a few systems and analyse them in detail and second, the need to overcome the geneticists' inability to obtain recombinants between structural and regulatory loci within a tightly linked gene complex. Such recombinants will be essential to relate different DNA sequences to changes in phenotype. □

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Are QSO absorption lines extrinsic?

from Derek Wills

THE observation of quasi-stellar objects (QSOs) in the ultraviolet (UV), made possible by rocket and satellite developments in the past few years, promises to help decide between alternative explanations of some properties of these still puzzling objects. One of the latest developments is that improved measurements of 3C273 in the UV lend support to the hypothesis that the absorption lines in the spectra of this and other QSOs are due to intervening galaxies (or other material) and that they are not intrinsic to the QSOs themselves.

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The first QSO whose redshift was determined is the radio source 3C273, which was observed spectroscopically by M. Schmidt (*Nature* 197, 1040; 1963). Although more than 1,500 QSO redshifts are now known, 3C273 is still the brightest (magnitude 13) and has almost the smallest redshift (0.16). Observations of 3C273 in the UV region are of particular interest because, at this relatively small redshift, it is possible to examine a spectral region that is seen from the ground only in QSOs at much larger redshifts. The result is a direct comparison of physical conditions in objects at quite different cosmological distances and, thus, at different cosmological epochs. Study of the UV

*Workshop on the Molecular Genetics of the Mouse II held at Sandbjerg Slot, Sønderborg, Denmark, on 11-16 August, organised by J. Peter Hjorth of the Institute of Ecology and Genetics, University of Aarhus and supported by the Danish Natural Science Research Council and Gl. Bomholtgaard Ltd. The abstracts will be published in *Hereditas*.

absorption-line spectrum also makes it possible to probe the medium between 3C273 and the Earth.

The first UV observations of 3C273 were made from a rocket (A. Davidsen *et al.* *Nature* **269**, 203; 1977) and were soon followed by broad-band UV measurements from the ANS satellite (C.-C. Wu *Astrophys. J. Lett.* **217**, L117; 1977). Not surprisingly, 3C273 was one of the small number of extragalactic objects observed during the commissioning phase of the International Ultraviolet Explorer (IUE) satellite by a team of twenty-six astronomers (A. Boksenberg *et al.* *Nature* **275**, 404; 1978), and two separate groups then obtained more detailed spectra of the QSO. One team published their results last year (A. Boggess *et al.* *Astrophys. J. Lett.* **230**, L131; 1979) and the latest contribution, by M.-H. Ulrich *et al.* (*Mon. Not. R. astr. Soc.* **192**, 561; 1980), is a collaborative effort by twenty astronomers at twelve institutions in five European countries. These spectra represent a total of about 16 hours of observing time with the 45-cm diameter telescope on board IUE, and their quality is comparable with those of spectra obtained by ground-based telescopes observing much fainter QSOs at larger redshifts.

It was clear from the first rocket spectra of 3C273 that its emission-line spectrum is indistinguishable from those of typical QSOs at large redshifts, and the details now reported reinforce this conclusion. The similarity is perhaps not as surprising as the fact that the emission-line spectra of QSOs at even the earliest cosmological epochs can be satisfactorily reproduced by simple models involving photo-ionization of a gas with chemical element abundances similar to those in the Sun. The underlying continuum of 3C273 can be represented fairly well by a power law over a large frequency range, except for a considerable excess of radiation in the UV-optical region. It was suggested by G.A. Shields (*Nature* **272**, 706; 1978) that this excess is attributable to thermal radiation from the vicinity of the black hole that is supposed to supply the enormous energy emitted by QSOs, and the observations of Ulrich *et al.* lend support to this interpretation of the UV-optical excess, in part by excluding some other suggested mechanisms.

The absorption-line spectrum of 3C273 is of particular interest. Virtually all large-redshift QSOs contain one or more sets of narrow absorption lines, usually at smaller redshifts than the corresponding emission lines. The strongest absorption lines are due to Lyman- α of hydrogen and other transitions in the UV, so that they are detectable from the ground only when the redshifts are large.

There is long-standing argument about the origin of these absorption lines. One interpretation is that they are caused by clouds of material ejected at high speeds (up to 0.1 – 0.5 of the speed of light), perhaps by the radiation pressure of the

QSO; another is that they arise in normal galaxies or intergalactic clouds that happen to intersect the very long path between us and the QSO. The two explanations are not mutually exclusive, although the minority of astronomers who still doubt the cosmological interpretation of QSO redshifts (see the recent review by G.R. Burbidge in *Nature* **282**, 451; 1979) cannot accept the second.

An obvious prediction from the ejection hypothesis is that absorption systems should be seen in nearby QSOs just as frequently as in the distant ones; even if, as in the early rocket observations, the wavelength resolution is not quite sufficient to detect individual, narrow absorption lines, the blending of lines below the wavelength of the Lyman- α emission should produce a noticeable drop in the continuum level in that part of the observed spectrum. On the other hand, if the 'intervening galaxy' hypothesis is valid, a relatively nearby object such as 3C273 would be unlikely to show displaced absorption lines or a drop in the continuum level below the Lyman- α .

No such change in the continuum level has been unambiguously seen in any of the UV spectra of 3C273, and the improved data from Ulrich *et al.* still show no evidence for individual absorption lines like those seen in high-redshift QSOs. Adherents of the ejection hypothesis will probably reply that observations of more than one low-redshift QSO are needed before their model can be ruled out, and that large ejection velocities (greater than about one-fifth that of light) would place the strong Lyman- α absorption below the IUE low-wavelength limit.

The latest data on the absorption lines in 3C273 also improve on the earlier evidence for absorption lines at zero redshift, arising in our galaxy. In particular, Ulrich *et al.* confirm the earlier IUE evidence (Savage and de Boer *Astrophys. J. Lett.* **230**, L77;

1979) for absorption due to CIV at 1,549 Å. This line indicates the presence of a highly ionized region at a temperature of $\approx 10^5$ K, and confirms the prediction (L. Spitzer *Astrophys. J.* **124**, 20; 1956) that our galaxy possesses a hot, gaseous corona, perhaps as a result of accretion of hot, intergalactic gas (D. Sciama *Nature* **240**, 456; 1972) or supernova explosions in the disk.

The presence of the CIV line in some of the absorption systems seen in high-redshift QSOs was formerly used as an argument against the 'intervening galaxy' model, on the grounds that there was no convincing evidence for such high temperatures and metal abundances in the haloes of normal galaxies. The detection of this spectral line in our own galaxy at roughly the intensity seen in the absorption spectra of high-redshift QSOs clearly removes this objection. Thus the presence of absorption lines at zero redshift and their absence at other redshifts must both be counted in favour of the idea that intervening galaxies similar to our own are responsible for many of the QSO absorption-line systems.

Undoubtedly, 3C273 will be among the first objects observed when the Large Space Telescope is launched in a few years. That eagerly awaited development will greatly increase the number of low-redshift QSOs whose UV spectra can be examined, perhaps leading to final agreement on the origin of QSO absorption lines and to a more detailed comparison of conditions in objects believed to have been formed at quite different cosmological epochs. In the meantime, the latest observations confirm that we can now use UV observations of QSOs as probes of the hot, outer regions of our galaxy — for even if there is still some lack of unanimity about QSO distances, there is at least agreement that they are outside our own galaxy. □

Biosynthesis and processing of cellular and viral polyproteins

from Edward Herbert

THAT active forms of polypeptides can be generated from inactive forms by proteolytic cleavages is an idea dating back to the discovery of precursor forms of gastrointestinal proteases (zymogens) in the early thirties. This type of activation is now known to occur with polypeptide hormones, proteases, blood coagulation factors and viral proteins. The activation process can be either an intracellular or extracellular event and is usually accomplished by trypsin-like enzymes.

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The discovery of viral and cellular proteins that serve as precursors to more than one biologically active entity has added a new level of complexity to the subject of protein processing.

At a recent conference*, scientists working on cellular and viral polyproteins were brought together to discuss similarities and differences in processing of these two classes of proteins.

Among the cellular proteins, pro-opiomelanocortin and proneurophysins

*Held in Hamburg, FRG in May 1980.

have attracted the most interest. Pro-opiomelanocortin, which contains 260 amino acids, is the precursor to adrenocorticotropin (ACTH), α - and β -melanocyte-stimulating hormones (MSHs), β -lipotropin (β -LPH) and β -endorphin [β (61–91)LPH]. The amino acid sequence of the precursor was worked out in 1979 by Nakanishi *et al.* (*Nature* **278**, 423, 1979) using recombinant DNA technology. This revealed that each biologically active domain in the precursor is preceded by a tryptic cleavage site (Lys-Lys or Lys-Arg). At the Hamburg meeting, A.C.Y. Chang (Stanford University) presented a partial sequence of the human pro-opiomelanocortin gene. One intron was found at a position 93 amino acids from the amino terminus of the protein but no other interruptions of the coding sequence were detected showing that genetic information for ACTH and β -LPH is contiguous. M. Chrétien (Montreal) presented the amino acid sequence of a 103-amino acid N-terminal fragment of the precursor isolated from the human pituitary. Sequencing work revealed two N-terminal amino acids (Trp and Arg) suggesting that there may be two pro-opiomelanocortin genes in humans.

Pro-opiomelanocortin is present in both anterior and intermediate lobes of mouse and rat pituitary (E. Herbert, University of Oregon). However, the precursor is processed to different end products in the two lobes. The major end products in the anterior lobe are glycosylated and unglycosylated forms of ACTH, β -LPH and N-terminal fragments of the precursor. In the intermediate lobe ACTH is processed further to α -MSH and β -LPH is processed to β -endorphin. The initial core glycosylation of the precursor and the first proteolytic cleavage appear to be the same in the two lobes, suggesting that processing differences in the two tissues arise at later stages. In the intermediate lobe of rat pituitary, β -endorphin [β (61–91)LPH] is rapidly converted to an N-acetyl derivative and a derivative missing four C-terminal amino acids (Zakarian, National Institute of Medical Research, London). These derivatives, which account for most of the endorphin in the intermediate lobe, have no opioid activity. In contrast to this situation, the major form of endorphin in the hypothalamus is β -endorphin.

Processing of pro-opiomelanocortin occurs quite early in the secretory pathway as subcellular fractions from rat pituitary enriched in endoplasmic reticulum and Golgi vesicles have a precursor/product ratio of 10. This ratio reaches a value greater than 100 in subcellular fractions enriched in secretory vesicles (Glombotski, University of Colorado). Loh (National Institutes of Health) showed that processing of pro-opiomelanocortin in the intermediate lobe of toad pituitary is similar to that in rat pituitary, and that inhibition of

glycosylation by tunicamycin leads to enhanced intracellular degradation of pro-opiomelanocortin to atypical peptides.

Considerable progress has been made in the past year in the study of the synthesis of neurophysins I and II. Three groups reported the synthesis of pre-pro-forms of neurophysins I and II in cell-free protein-synthesizing systems operating under the direction of hypothalamic mRNA (Chaiken, National Institutes of Health; Richeter, University of Hamburg; McKelvy, University of Pittsburgh). Pre-pro-neurophysin I has a molecular weight of 17,000–19,000 and contains an antigenic determinant for oxytocin. Pre-pro-neurophysin II has a molecular weight of 23,000–25,000 and contains an antigenic determinant for vasopressin (Richter). Peptide mapping studies confirm the presence of the neurophysin sequences in pre-pro-neurophysins I and II but sequences of vasopressin and oxytocin could not be detected (Chaiken). The possibility that still higher-molecular-weight forms of neurophysins exist was raised by P. Cohen (University of Paris) who fractionated hypothalamic proteins by gel filtration in denaturing solvents. A protein moiety of molecular weight greater than 50,000 was reported to contain antigenic determinants of neurophysins I and II and oxytocin and vasopressin.

Do these findings about polypeptide precursors of hormones and other biologically active entities have any relevance to what has been found for viral polypeptides? Host cell proteases are most likely involved in very early steps (initial translation of a polypeptide) or in very late steps (transport through membranes or at maturation) of viral replication. In addition, proteolytic enzymes are synthesized by many viruses that are responsible for very specific steps in protein processing. Finally, interesting modifications of viral proteins take place as prerequisite for or as the result of function.

For example, the initial cleavage of the polypeptide of picornaviruses (molecular weight 220,000) is likely to occur by a host cell protease. Such function could reside in the 80S ribosome (Korant, University of Wilmington). One of the initial cleavage products of the polypeptide of encephalomyocarditis virus (a picornavirus), on the other hand, is capable of autocatalytic cleavage yielding a virus-specific protease, p24, which in turn processes other viral proteins (Rueckert, University of Cleveland). In contrast, the precursor protein Pr76^{gag} of avian retroviruses is incapable of autocatalytic cleavage, although its C-terminal portion (p15) is a virus-specific protease. p15 must be generated first by a host function before it can proceed to process the rest of the viral precursor protein (Eisenmann, University of Washington; Dittmar, University of Berlin). Both p24 of encephalomyocarditis virus and p15 of retrovirus have a narrow

range of specificity but a definition of their action must await further studies on the structure of viral proteins.

Late reactions in viral maturation taking place as the virus buds from the cell in the completed viral particle were also discussed. Evidence was presented for the involvement of several proteases with unique specificities in cleavage of murine leukaemia virus proteins during viral morphogenesis (Luftig, University of Ohio). One activity exhibited characteristics of serine proteases, another hydrolysed two X-Pro bonds (with X being either tyrosine or phenylalanine). The haemagglutinin of influenza virus also has to be hydrolysed at a critical site to expose a new amino-terminal region which is crucial for infectivity (H.-D. Klenk, Giessen). This cleavage requires a trypsin-like enzyme which must be supplied by the host. Other proteases may cleave adjacent peptide bonds but this does not yield infectious viral particles. A similar example has been described in the maturation of Semliki Forest virus — in cells infected with this virus, a 26S mRNA is produced which codes for a polyprotein cleaved into capsid protein and two transmembrane proteins. The nucleotide sequence of the 26S mRNA has recently been completed (H. Garoff, Heidelberg) and it was found to contain an uninterrupted reading frame which can code for more than 1,200 amino acids. In conjunction with data on the termini of mature virus proteins, the cleavage sites, membrane-spanning segments and putative signal sequences can be discerned. Interestingly, the last cleavage of this polyprotein, the extracellular processing of the transmembrane protein p62 to yield proteins E2 and E3, again involves a trypsin-like specificity.

Viral glycoproteins undergo cleavage by host cellular signal peptidase while being transported to the cell surface. The value of viral proteins as probes to study modification and transport of polypeptides was documented by Lodish (University of Cambridge), who also discussed the interesting phenomenon of fatty acid acylation of certain cellular and viral glycoproteins. Another novel modification of polypeptides is the polyadenylation diphosphate ribosylation of some reovirus capsid proteins (Carter, State University of New York), a phenomenon that may play a role in regulating events in reovirus morphogenesis. Finally, all newly synthesized viral RNA of poliovirus is covalently linked to a small protein, VPg, at the 5' end via a O⁴-(5'-uridylyl)-tyrosine bond (Wimmer, State University of New York). VPg maps in the precursor (NCVP1b) for the viral RNA polymerase as well as viral protease (Rueckert; Wimmer). Since no free VPg can be detected in the infected cell, initiation of RNA synthesis may be linked to a step of proteolytic cleavage of the precursor for the viral RNA polymerase, a situation unique among animal RNA viruses. □

Lessons from a peptidergic neurone

from B. T. Pickering

SECRETED polypeptides are known to begin their lives as parts of larger precursors but for many biosynthetic pathways the question persists of 'how large is larger'. This question has recently been asked for the precursors of vasopressin by Paul Cohen and his associates in Paris (*Proc. natn. Acad. Sci. U.S.A.* 77, 2587; 1980). They have found evidence, primarily based on Sephadex chromatography in the presence and absence of urea, of a large ($\approx 140,000K$) precursor molecule in beef posterior pituitaries which showed immunoreactivity with both antivasopressin and antineurophysin sera.

The posterior pituitary hormones were the first recognized members of the neuropeptides, a group which has been growing in both size and importance. Although the hormones oxytocin and vasopressin are released from the posterior pituitary, the gland consists essentially of a collection of the ends of nerve fibres whose neuronal cell bodies are located in the supraoptic and paraventricular nuclei of the hypothalamus. Protein synthesis occurs only in the cell body but the synthetic product is packaged into granules and transported along the nerve fibres for storage in, and release from, the posterior pituitary gland. Along with the nonapeptide hormones, the gland contains a family of polypeptides with molecular weights around 10,000 — the neurophysins. These molecules form strong ionic complexes with the hormones with a pH optimum of 5.5, close to that inside the secretory granule. It is not surprising, therefore, that neurophysins have been regarded as carrier proteins necessary to keep the hormones inside the granules during their intra-axonal journey.

The hypothesis that vasopressin and its neurophysin share a common precursor was first put forward by Howard Sachs in the mid-sixties. Sachs and Takabatake

(*Endocrinology* 75, 943; 1964) presented the first real evidence for post-translational processing of a polypeptide hormone precursor and the group went on to include neurophysin in the precursor, summarizing their views at the same Laurentian conference that received Steiner's evidence for the existence of proinsulin (*Recent Prog. Horm. Res.* 25; 1969). The hypothesis became much more plausible when it was realised that almost all species elaborate at least two neurophysins — one which is biosynthetically related to vasopressin and another related to oxytocin. Other minor neurophysins seem to be metabolic products of these two (see *Ann. N. Y. Acad. Sci.* 248; 1975).

Undoubtedly the major breakthrough in this field, which provided some hard data to back up the circumstantial evidence, came from Hal Gainer and his group at the US National Institutes of Health (*J. Cell Biol.* 73, 366; 1977; *Science* 207, 373; 1980). In brief, the Gainer group has used pulse-chase studies to show that the two major neurophysins of the rat are formed from two precursors ($\approx 20,000K$) and that processing continues within the secretory granule while it is travelling along the axon. Although the NIH group has not been able to demonstrate conclusively that the precursors do contain hormone as well as neurophysin sequences within their molecules, they have shown that the putative vasopressin precursor is absent from animals unable to make vasopressin (Brattleboro rats). Also, after limited tryptic digestion, the precursor produces molecules with characteristics similar to vasopressin as well as to neurophysin.

The recent paper of Cohen *et al.* (*op. cit.*) reports evidence for a similar ($\approx 25,000K$) precursor in beef pituitaries and this did have immunological determinants for both neurophysin and vasopressin, as did the larger ($\approx 140,000K$) component.

Moreover, protease activity generated molecules with the characteristics of the fully processed secretory products. Similar 20,000–25,000K neurophysin precursors have been synthesized in cell-free systems using mRNA extracted from the supraoptic nuclei of cattle by Guidice and Chaiken (*Proc. natn. Acad. Sci. U.S.A.* 76, 3800; 1979) and of mice by McKelvy and colleagues (*Biochem. biophys. Res. Commun.* 89, 943; 1979). The neurophysin (10,000) and hormone (1,000) sequences would account for about 11,000 of the precursor molecular weight, leaving some 10,000 to be accounted for, and Gainer's group has evidence that in the putative vasopressin precursor this is represented by a 10,000K glycopeptide.

Thus the 20,000–25,000K precursor can be readily accounted for in terms of processed products and has an extractable mRNA to direct its synthesis. What constitutes the residual 110,000 in the larger molecule? Does it form part of the biosynthetic pathway? Certainly there seem to be comparable amounts of 140,000 and 25,000K forms remaining in the granules when they reach the posterior pituitary gland where processing is all but complete (96%). If the 140,000K form is an early stage of the usual pathway, the products of the residual 110,000 should be present in the secretory granule in amounts equimolar with neurophysins and hormones. If not, it must follow that the 140,000K form is a polymer of the 25,000K precursor, perhaps coming from the translation of a polycistronic message; such a possibility is allowed by the Paris workers. The solution to this problem, along with the exclusion of aggregation arising from disulphide interchanges, must await a re-examination of pulse-chase studies in the hypothalamus where precursor/product ratios are more favourable than in the posterior pituitary.

Quite apart from the size of the primary translation product, in my view (*Essays in Biochemistry* 14, 45; 1978) the great attraction of the hypothalamo-neurohypophysial system is that it provides a good model system for studying polypeptide secretion. Its constituent neurones show all the properties of secretory cells but there is a division of labour (see Fig. 1) such that synthesis and packaging take place in the perikaryon, transport of granules occurs along the axon, and storage and release of the product is restricted to the nerve terminal. This permits the constituent phases of secretion to be studied in some degree of isolation one from another, leading, for example, to the evidence that processing continues while the granule is in

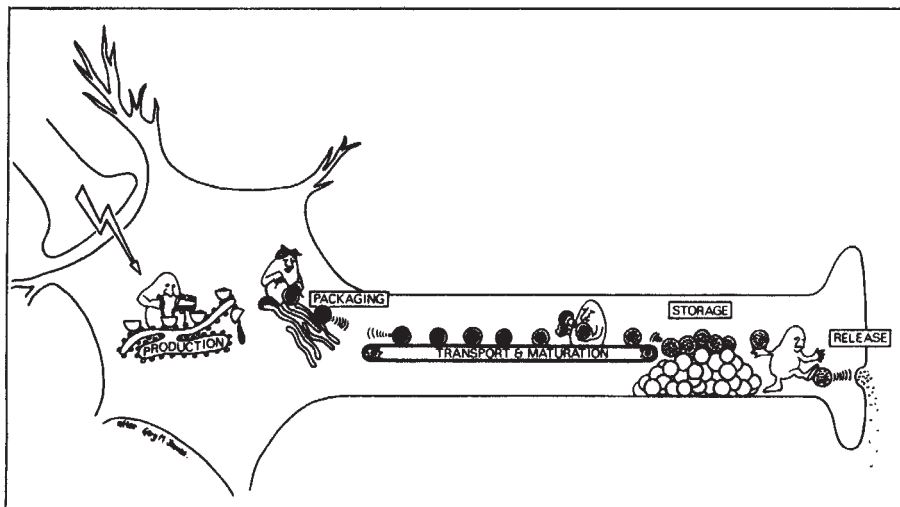


Fig. 1 The division of labour in a peptide-secreting neurone. (After an idea by N.McC. Mortensen.)

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transit. The peptidergic neurone as a model system was the dominating theme of the recent 8th International Symposium on Neurosecretion*. Also at this meeting, H.-D. Dellman (Iowa) showed that, in the developing neurone, secretory material appears within the cisternae of the endoplasmic reticulum before the appearance of Golgi complexes or secretory granules. It is tempting to relate this observation to the almost simultaneous report of A. G. Robinson (Pittsburgh), at another meeting†, that while immunoreactive neuropeptin appeared in the brains of fetal

rats on day 13 of gestation, peaked on day 14 and fell slowly towards term, the hormones were undetectable on day 13 but their content in the brain increased towards term. If, as Robinson suggests, these results reflect a high relative concentration of precursor forms in the third fetal week, then, taken together with Dellman's findings, they suggest that the developing hypothalamo-neurohypophysial system presents a tool for the study of the role of the Golgi complex in precursor processing. □

*Neuropeptides, development and ageing; held at Rochester, New York on 3–5 September 1980.

*Held at Friday Harbor, Washington on 4–10 September 1980.

The search for the Zvolen fireball

from David W. Hughes

FIVE all-sky camera stations of the European Fireball Network recorded a bright fireball which penetrated low into the atmosphere on 27 May 1979 at 20h 39min. The fireball terminated close to Zvolen in central Slovakia and may have produced several meteorites.

Geometric, dynamic, photometric and orbital data have been calculated by Z. Cepelach, J. Boček and M. Ježková of the Astronomical Institute of the Czechoslovak Academy of Sciences, Ondřejov, V. Porubčan of the Astronomical Institute of the Slovak Academy of Sciences, Bratislava and G. Polnitzky of the University Observatory, Vienna. Their results have been published in a recent edition of the *Bulletin of the Astronomical Institutes of Czechoslovakia*

31, 176; 1980.

The photometric and positional data on all the available photographic records were measured using a Zeiss Ascocord device. Computational analysis gave the light curve shown in Fig. 1. It is interesting to note that the magnitude remained reasonably constant during a considerable portion of the trajectory. The luminous power is approximately proportional to the kinetic energy lost per unit time by the incident body and it can be seen that mass loss dominates the constant luminosity phase. The maximum deceleration occurs well inside the luminous trajectory and this is typical for meteorite-dropping fireballs. The end height, coupled with the initial mass, velocity and trajectory, indicates that the meteorite was an ordinary

chondrite (a 'strong' stone). The low initial velocity is responsible for the maximum luminosity occurring between heights of 60 and 30 km. Two of the photographs showed the separation of three fragments from the main trail. Fragmentation occurred at heights of 28, 33 and 40 km.

The computational analysis also gave the orbit of the incident meteorite. This had a perihelion distance of 0.89 AU (just inside the Earth's orbit), an aphelion distance of 2.84 AU (right in the asteroid belt) and an inclination to the ecliptic of only 2.3°.

The mass of the final falling meteorite and fragments was estimated to be a kilogram or so. The statistics of meteoritic events from fireball camera networks in the American and Canadian Prairies, demonstrate that one observable meteorite fall per year should occur within a network area of about 1.4×10^6 km². As the cameras can only register fireballs efficiently when the sky is completely dark, the influx rate of meteorites will be about three times higher than this figure. This is equivalent to about 1,100 meteorite falls per year over the whole surface of the Earth and about 300 over the land masses. In the regions where the networks are active it has been found that the searches that follow the observed falls only have, on average, a 10 per cent chance of recovering the meteorite. Under these assumptions, the Slovakian part of the European network should observe a meteorite fall every 30 years and recover one every 300 years.

The search area for the Zvolen meteorite was estimated to be about 10 km². This is larger than usual. The photographic station which predicts the fall site most accurately is the one closest to the terminal point of the luminous trail. The camera at Močiar was only 15 km from the predicted position but the gremlins that haunt all scientific endeavour were at work. The Močiar camera was opened on that evening two minutes after the apparition of the fireball. The central part of the search area lies in the eastern part of the town of Zvolen, a region containing many urban dwellings, two large factories, a railway depot with piles of coal and a small river that is swampy in parts. The other regions of the predicted fall area are hilly and covered with woods, fields and meadows. The possibilities of finding a strange, 1-kg rock, about 10-cm across, do not seem too great.

Great publicity was given to the event in the hope of obtaining eye-witness reports of the fireball flight near the impact point. It was surprising that only seven reports were forthcoming in a region of such dense population and only two reports helped to reconstruct the whole path of the flight.

Well over a year has passed, including an early spring when vegetation is much thinner than usual. Nothing has yet been found. □

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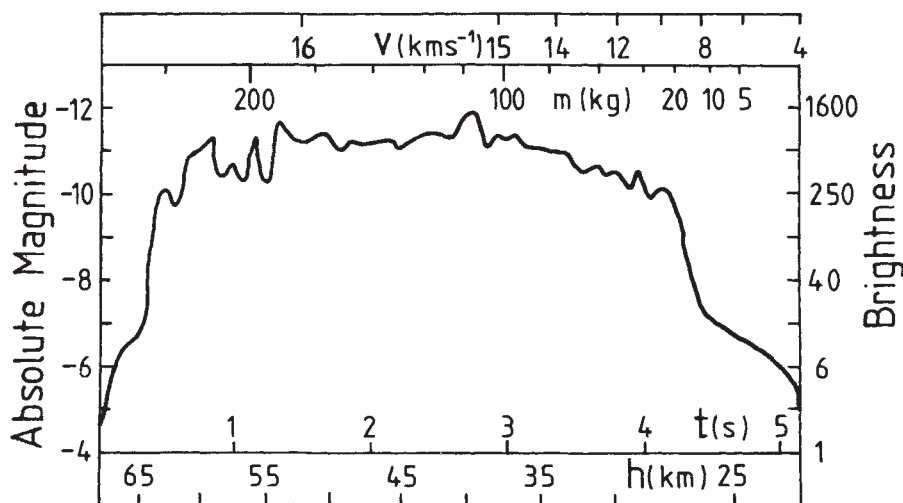


Fig. 1 The light curve of the Zvolen Fireball over the brightest part of its trajectory. The left ordinate gives the absolute photometric magnitude, which is the magnitude the fireball would have if it was at a standard position 100 km from the observer at the zenith. The right-hand ordinate gives the brightness, standardized so that a brightness of 1 arbitrary unit is equivalent to an absolute magnitude of -4. The main abscissa is time, measured in seconds. Secondary abscissae show the decrease in the height of the causative meteorite and also its mass loss and deceleration. At $t = 0$, the meteorite had a calculated mass of 223 kg and a velocity of 16.12 km s⁻¹.

REVIEW ARTICLE

The diffuse UV background

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The diffuse radiation field in the UV (900–3,000 Å) affects the structure of galactic molecular clouds and conveys important information concerning the physical characteristics and spatial distribution of gas and dust in the Universe. Continuum emission in this range is probably dominated by interstellar dust scattering in our Galaxy. For view directions and angular resolutions allowing observations in the rifts between galactic dust clouds, the background due to the integrated light of spiral galaxies may be detected, providing important information on their structure and evolution. The redshifted emission from an intergalactic medium may be observable in the regions between nearby bright galaxies. Present observations provide weak constraints on the radiation field required to ionize the intergalactic medium at the level required by the Gunn–Peterson test.

THE recent enhanced capabilities of UV instrumentation and observing platforms, a better understanding of basic emission processes coupled with rapid progress in associated fields (especially dust grain characteristics and distribution and galactic luminosity functions) have contributed to a rapid growth of diffuse UV astronomy. UV spectra of galaxies from the OAO, IUE and ANS satellites have enabled their contribution to a diffuse background in the UV to be assessed. New optical observations of hot objects and of high latitude reflection nebulae have become useful for probing the origin of the observed UV radiation field and assessing the likelihood of reaching extragalactic sources.

These factors make the present situation more complex than that envisaged by Davidsen *et al.*¹, but there are still conflicting observations indicative of the fundamental difficulty of observation. We attempt here, using these latest findings, to determine as accurately as possible the characteristics of the most likely sources of the diffuse UV background. We have placed special emphasis on assessing the probability that information of cosmological significance can be gleaned from this work. Galactic dust and external galaxies are likely to have an important role. These sources must be well understood before the intergalactic medium contribution, if any, can be properly quantified especially if it is emitting by recombination radiation rather than collisional excitation as has been assumed in the past.

Local sources

The most serious difficulty in analysing diffuse cosmic background data from UV instruments is to account for the large number of sources that may contaminate the observations. The inherent instrument background is not as easily removable for diffuse emission spectrophotometry as it is for point sources. Shutters that can exclude the outside world periodically are the only reliable method, but complex moving parts in spacecraft instrumentation are not always considered ideal. Pointing the instrument at some dark object is a common alternative but the actual darkness of the object is questionable. Dark Earth may not be so dark at certain altitudes and latitudes. In fact, it may be brighter than the sky due to various electromagnetic or particle induced emissions in the UV.

This atmospheric emission—another source of diffuse radiation—must be fully understood when observing from an Earth orbiting platform. There are at least 20 known or suspected UV line and band emission features in the Earth's airglow and aurora. Particle induced emissions are probably the most

treacherous of these sources because of their intensity and uncertain spatial and temporal variation over the Earth's surface. This source is not restricted to the auroral zones, but may appear almost anywhere at any time, including the equatorial regions due to ring current precipitation^{2,3}. It is essential to exclude at least the most intense emission lines from the instrument's bandpass. However, this may not be a sufficient precaution as weak band emissions throughout the far UV may contribute to a broad band observation⁴.

A more drastic solution would be to escape the Earth's atmosphere and observe from a platform in the interplanetary medium. However, even this medium is a source of diffuse UV radiation. In this case, scattering of solar light by zodiacal dust grains and by atomic and singly ionized interstellar gas atoms must be considered. The former source of emission is probably not important below about 2,000 Å (refs 5, 6) but becomes troublesome just above this wavelength. At an elongation of 30° on the ecliptic plane, the zodiacal light intensity at 2,000 Å may reach ≈ 500 photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}^{-1}$, a value uncomfortably close to the observed cosmic background intensities plotted in Fig. 1. Any correction for this effect should include systematic exploration of the ecliptic plane at many elongations and observations as far removed from this plane as possible.

Backscattered UV line radiation at the resonance transitions of interstellar hydrogen and helium flowing into the interplanetary medium because of the large relative motion of the Solar System with respect to the interstellar medium is also of considerable importance⁷. Observed emissions include the H I 1,216 Å and 1,026 Å and the He I, 584 Å resonance lines with suspected weaker contributions from He II 304 Å, He I 537 Å, N II 1,085 Å, O I 1,304 Å, and C II 1,336 Å that have the necessary corresponding exciting lines in the solar spectrum. These emission lines influence UV observations by their strength (especially Ly α at $\approx 3 \times 10^8$ photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1}$) and seasonal variability.

Point sources

Progress in understanding point sources of UV emission critically influences the results of the UV background observations because some of these sources will be in the field of view of an instrument attempting to measure the diffuse background. The best way of correcting for stellar contamination is to use the TD1 catalogue of stellar UV fluxes⁸ covering the range 1,500–2,500 Å down to $m_v \approx 8-9$, supplemented, where possible, by the corresponding spectrophotometric catalogue^{9,10}. For this technique to be accurate enough, the instrument response and

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the field of view must be well known, preferably by means of careful in-flight calibrations.

For fainter stars or for $\lambda < 1,500 \text{ \AA}$ we have to use theoretical models for the stellar emission and luminosity functions for hot stars obtained in the optical band; large errors are, therefore, to be expected.

Henry¹¹ has described a technique to overcome some of these problems in which stars in the SAO catalogue are integrated in conjunction with a UV stellar calibration¹². Although simple, this method may overestimate the stellar contribution due to errors in the listed spectral types and the lack of a suitable correction for differential interstellar dust extinction.

A simpler method, in principle, is to reduce the instrument field of view to a size such that the number of stars having a certain limiting magnitude becomes very small. This limiting magnitude has been estimated for observations at $1,500 \text{ \AA}$ to be near $m_v = 9.5-10$ (ref. 11). This is effective provided the collecting area of the telescope rises simultaneously as the square root of the decrease in the field of view to maintain the same signal-to-noise ratio; this may become impractical or too expensive. As a rule, with recent technology, a 1-m diameter telescope is necessary to achieve a sensitivity slightly below that needed to detect the lowest observed UV intensity ($\approx 300 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$ at $1,500 \text{ \AA}$) with a 15–20 arc min field of view.

An added advantage can be envisaged by restricting one's background observations to high galactic latitudes where hot stars are few and far between. This works well for O and B stars, is dubious for A stars and of no use for F and later stars. Because A stars (especially A0 stars) contribute substantially to the background above $1,500 \text{ \AA}$ (ref. 11), this technique is best suited to observations in the range $\approx 1,000-2,000 \text{ \AA}$.

After these precautions, there remains the important question of the residual faint ($m_v > 10$) star distribution and the possible magnitude of its contribution to the observed UV background. We have estimated this contribution using standard main sequence colours, absolute visual magnitudes, scale heights and luminosity functions. Conversion to the UV is accomplished by using observed mean $(m_v - m_\lambda)_0$ versus $(B - V)_0$ relations^{12,13}, with the result that the contribution due to the sum of all main sequence stars of $m_v > 10$ in the Galaxy at $1,500 \text{ \AA}$ in the direction of the galactic poles cannot exceed $30 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$.

Not all hot stars, however, can be assumed to be on the main sequence. Green¹⁴ has shown that hot white dwarfs comprise $\approx 64\%$ of the known faint blue star population down to a B mag of ≈ 16 . Using his derived luminosity function and scale height for these objects and assuming that all the hot white dwarfs in his sample are as hot as HZ43, one of the hottest white dwarfs known¹⁵ we can calculate a strict upper limit to their effect on an UV background measurement. We find that their contribution at the poles and at $1,500 \text{ \AA}$ cannot exceed $\approx 20 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$. Other possible sources of stellar UV radiation such as subdwarf OB stars and blue horizontal branch stars are probably too scarce in space to give a substantial contribution¹⁶. Thus with the accuracy allowed by the input astronomical data, unresolved faint stars will contribute $< 50 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$ at $1,500 \text{ \AA}$ in the direction of the galactic poles.

Observations

All observers have to deal with at least some of the above problems to claim a measurement of a truly cosmic (galactic or extragalactic) background. Henry *et al.*¹⁷, using a rocket-borne $10^\circ \times 10^\circ$ Geiger counter sensitive to the $1,425-1,640 \text{ \AA}$ range, mapped the entire sky observable in March from White Sands, New Mexico. After in flight calibrations and subtraction of the average count rate observed when the detector was pointed 25° or more below the horizon were performed, the residual signal was compared with the signal expected from the integration of the stars in the SAO catalogue. The data for $b < 45^\circ$ are reasonably well accounted for by the predictions but a large observational excess is evident in the range $b > 45^\circ$. At high galactic latitudes Henry *et al.* find the intensity of the UV background corrected for stellar contributions to be in the range $1,000-1,900 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$. The high galactic latitude measurement is displayed in Fig. 1 where we have plotted the observed background intensity in units of $\text{eV cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ eV}^{-1}$ as a function of wavelength in $\text{\AA}$ for each measurement discussed here. According to Henry *et al.*, the reported excess is due to airglow in the $1,425-1,640 \text{ \AA}$ band and one may hypothesize the presence of weak emissions of N_2 in the Lyman-Birge-Hopfield bands⁴ as a possible source of this contamination.

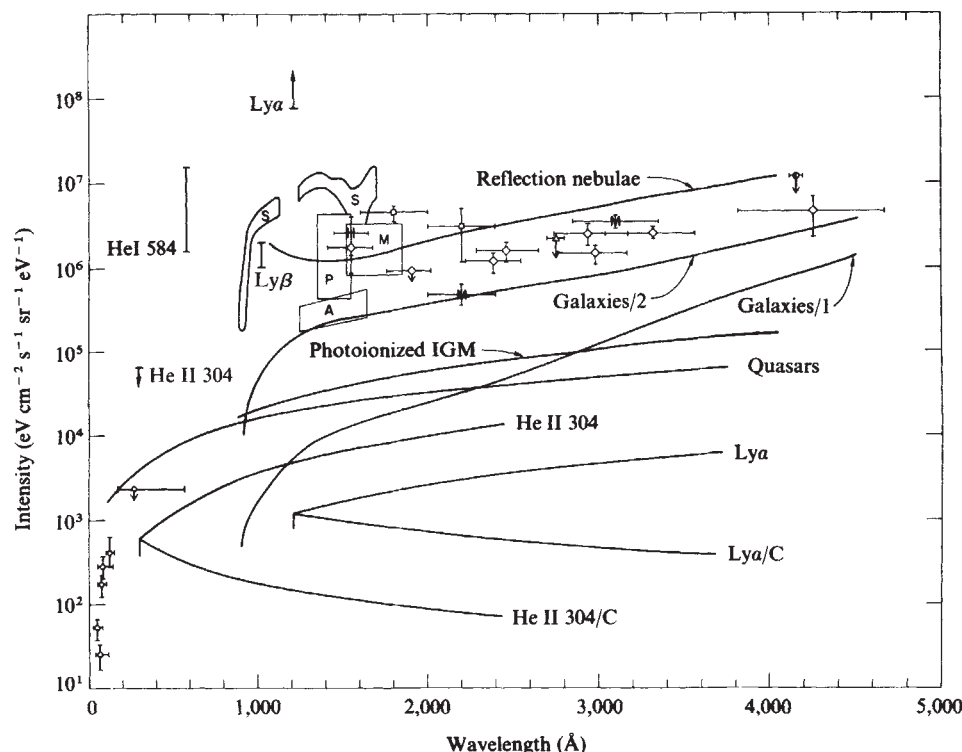


Fig. 1 Observations and theoretical estimates of the diffuse UV background at high galactic latitudes as a function of wavelength (delete eV^{-1} for the interplanetary lines). Sources of the observations: A, ref. 22; H, ref. 17; $\diamond$, ref. 18; M, refs. 24, 28; $\triangle$, ref. 29; P, refs. 25, 26; $\circ$, ref. 34; $\square$, ref. 23; S, ref. 31; $\otimes$, ref. 35.

Lillie and Witt¹⁸ used the Wisconsin Experiment Package 10×10 arc min broadband photometers on OAO 2 to study the intensity, spectrum and spatial distribution of the UV background between 1,550 and 4,250 Å. Their results are summarized in Fig. 1 which displays their average intensities in the $40^\circ < b < 90^\circ$ range. Their S_{10} units are converted to our physical units using the TD1 spectrum of α Lyr⁹. These results supersede the earlier published observations from OAO 2 (ref. 19) because of their superior accuracy, spatial coverage and signal-to-noise ratio.

The OAO 2 measurements plotted in Fig. 1 are corrected for instrumental background using mechanically actuated shutters, for zodiacal light by means of an empirical correction formula incorporating several observational results and for light of stars of $m_v > 11.5$ by optical photoelectric drift scanning of the observational fields. For the reasons outlined above we would expect the most accurate numbers in this case to be those corresponding to the shortest wavelengths and to the highest galactic latitude range observed. In this case, however, the weakness of the signals in the very small OAO field of view at high galactic latitudes introduces a substantial observational error that may approach 100% of the observed signal in some cases. The fact that the adopted zodiacal light spectrum below 2,000 Å (ref. 20) may be too high⁶ probably has very little influence on the results because of the vanishing solar flux at 1,500 Å.

With a $12^\circ \times 12^\circ$ scanning spectrometer sensitive to the 1,180–1,680 Å range, Henry *et al.*²¹ observed the sky near the south and north galactic poles from Apollo 17 on trans-Earth coast, free from the effects of the terrestrial atmosphere. The resulting UV background spectrum consisted of a wide emission feature centred at 1,450 Å, of maximum intensity ≈ 250 photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}^{-1}$ and with little or no emission at 1,250 and 1,650 Å. Unfortunately, a large and complex correction for instrumental background, starlight and Ly α scattering in the instrument amounting to $\approx 85\%$ of the signal significantly reduces the confidence level of the result.

In a repeat experiment performed from a rocket by Anderson *et al.*²², the 1,450 Å 'feature' at the galactic pole disappears and is replaced by a featureless constant background of ≈ 285 photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}^{-1}$ between 1,250 and 1,650 Å. A suggestion of a ledge or discontinuity at $\approx 1,650$ Å is found in this experiment which, because of its smaller field of view ($1.4^\circ \times 5.8^\circ$) is less susceptible to large starlight subtraction errors. The outer envelope of the spectrometer results from this experiment is plotted in Fig. 1 rather than the results from Apollo 17 as the latter are far more uncertain and highly discrepant with the rocket results at 1,250 and 1,650 Å. The former should be considered essentially free of the problems encountered by the Apollo 17 experiment because the instrument background was low, a crystal filter shielded the instrument from Ly α radiation and the stellar correction for two of the three targets observed was quite small. From the results on these three targets, Anderson *et al.* suggest that the UV background is spatially uniform at this intensity.

A three channel UV polychromator with a 2° circular field of view flown on a rocket by Pitz *et al.*²³ measured the UV sky brightness at 1,800, 2,200 and 2,600 Å. After the data were purged of instrumental effects and corrected for airglow and zodiacal light and starlight contaminations, the intensities for $b = 60^\circ$ displayed in Fig. 1 were reported. The quasi twilight observing configuration (solar zenith angle $\sim 110^\circ$) makes correction for airglow risky. Pitz *et al.*²³ claim that the brightness distribution with view direction is uniform but it is not clear at what level of accuracy. Only a few observations at different latitudes are reported—the result of an extensive averaging procedure that might easily mask a more pronounced spatial variation.

A similar difficulty of interpretation is encountered with data of Maucherat-Joubert *et al.*²⁴ obtained from the ELZ photometer on board the D2B satellite. This experiment consisted of a $1.3^\circ \times 1^\circ$ objective grating-photomultiplier detector

combination sensitive to the 1,525–1,855 Å range surveying the entire sky from a 600-km altitude orbit. The outer envelope of the validity range of their results for $b > 40^\circ$ is shown in Fig. 1. Because of the altitude of the satellite, the wavelength band, the small field of view and the observing configurations, airglow, zodiacal light and starlight probably form a small part of the observed signals after elimination of the bright star fields ($m_v > 8$). Because the instrumental background was high and difficult to assess properly, the data was averaged over relatively large spatial zones, thereby losing possible spatial information. As the observed range of intensities was much wider than expected from counting statistics alone, an additional source of emission was invoked. Faint stars with the true UV background consequently uniform were suggested in accordance with the results of Anderson *et al.* and Pitz *et al.* Another possibility is that the spread is due to an actual spatial variation of the UV background itself.

The latter conclusion would be more compatible with the results of the survey carried out with the 2.5° field of view telescope on board the Apollo-Soyuz Test Project (ASTP). A patchy spatial distribution of the UV background is reported^{25,26} with intensities ranging from a sharp minimum of 300 to $\approx 4,000$ photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}^{-1}$ after correction for stars of $m_v \leq 9$ –10, instrument background and airglow. The latter corrections were made using a moveable shutter and observation of the Earth from the 225 km altitude of the spacecraft. The range of intensities observed for $b > 40^\circ$ is also shown in Fig. 1 at the appropriate wavelength band.

There are two obvious explanations for the radiation pattern observed: (1) diffuse radiation scattered by galactic dust or (2) random airglow contamination not accounted for by the Earth pointed observations and not satisfying the standard rules to airglow phenomenology²⁷. The latter explanation of the discrepancy between the Paresce *et al.* results and those of Anderson *et al.* is the simplest because, for at least two of the Anderson *et al.* targets, there corresponds an ASTP observation made in essentially identical conditions and yielding a factor of ≈ 3 difference in intensities. However, the clear correlation found²⁶ between the ASTP UV background intensity and galactic N (H I) column density as determined by 21-cm radio observations suggests a galactic origin of a good part of the observed signal.

This result has been confirmed by Maucherat-Joubert *et al.*²⁸ after a reanalysis of the D2B data. They constructed histograms of individual count rates corresponding to areas in the sky of 24–40 square degrees, regions much smaller than those considered in the original analysis. After removal of resolved stars, subtraction of dark current and zodiacal light, a reasonably good correlation between observed 1,690 Å intensity and N (H I) is found for all view directions corresponding to $b > 40^\circ$. The large scatter in the correlation may be partly due to variations in galactic plane brightness and dust-to-gas ratio from point to point in the sky²⁶.

Further analysis²⁸ of the UV background with the ELZ instrument at 2,200 and 3,100 Å is reproduced in Fig. 1. The similarity in shape of the UV background spectrum towards the poles and towards the Coal Sack nebula has been used to favour the scattered galactic light hypothesis.

Morgan *et al.*^{29,30} have reported TD-1 measurements of the UV background. Unfortunately, because of substantial airglow contamination (especially Ly α) of uncertain magnitude, only the variation of the signal with galactic latitude could be ascertained with any accuracy. Thus these results have limited value except to confirm that the albedo of interstellar grains is ~ 0.5 in the 1,550–2,740 Å range and to set an upper limit to the UV background at 2,740 Å which is shown in Fig. 1.

The UV spectrometer on the Voyager spacecraft³¹ has the capability of measuring the UV background in the 500–1,700 Å region with ≈ 10 Å wavelength and $0.9^\circ \times 0.1^\circ$ spatial resolution with high sensitivity due to the long observing times available and the absence of terrestrial airglow contamination. Large instrumental backgrounds, uncertain corrections for Ly α

interplanetary emission and uncertainties in instrument calibration still hamper attainment of the ultimate instrument sensitivity to weak diffuse fluxes.

These problems necessitate subtracting a background spectrum obtained in one view direction and used as a 'scattering background spectrum' from a spectrum obtained in another direction as was done in the Voyager results analysis. Hence spatial variations conveying basic physical facts concerning the radiation field may be obliterated. The outer envelope of the results of this experiment are shown in Fig. 1. The generally higher values may, in part, be due to the generally lower galactic latitudes ($b < 40^\circ$) of the Voyager view directions than for the other measurements displayed in Fig. 1 which generally pertain to the $b > 40^\circ$ region.

The spectral feature at $\approx 1,450 \text{ \AA}$, first detected by Henry *et al.*²¹ and not confirmed by Anderson *et al.*²², is also reported by Hua *et al.*³² but of varying intensity in the two places in the sky observed. This experiment consisted of a $53 \text{ \AA}$ resolution, $6^\circ \times 6^\circ$ field of view scanning spectrometer sensitive to the $1,100\text{--}1,900 \text{ \AA}$ region flown on the Prognoz-6 satellite. Because calibration of the instrument is uncertain it is premature to give it the same weight as the other measurements shown in Fig. 1.

The UV background is almost certainly made up of components displaying widely different physical characteristics in the emitted radiation field including different intensities, spatial variations and polarization at different wavelengths. Attempts to unify these properties into a diagram similar to Fig. 1 will probably turn out to be an oversimplification. Because of our limited knowledge, however, it is useful for gaining a general perspective on the problem that would be difficult if we were restricted to a small subset of the UV spectrum. It also ensures that any postulated source of UV radiation does not violate observational limits in the adjacent spectral regions. For these reasons we have expanded the scale of Fig. 1 to include the EUV on one end, the visible on the other, and have included results pertaining to different phenomena that must be present whenever a measurement of the UV background is attempted. It also vividly displays the variety of physical emission mechanisms operating in the UV and the information one can obtain concerning the structure of the interstellar gas and dust components.

Figure 1 thus includes observations of the EUV background in the $50\text{--}600 \text{ \AA}$ wavelength region^{33,34}, the range of observed intensities of the diffuse interplanetary emission lines at 304, 584, 1,026 and $1,216 \text{ \AA}$ and the limit on the night sky background at $4,150 \text{ \AA}$ (ref. 35). Although the scatter, individually and collectively, of the data is large, there seems to be remarkable agreement over a wide range of wavelengths from the visible to the beginning of the EUV that the UV background is relatively constant between 10^6 and $10^7 \text{ eV cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ eV}^{-1}$ at high galactic latitudes. The largest deviations occur in the far UV ($1,000\text{--}2,000 \text{ \AA}$) where most observations are concentrated. It is not yet possible to decide whether the large scatter at these wavelengths reflects reality or is due to the observational problems discussed above. If the scatter is real and due to variations in observing direction and wavelengths, for example, the relative lack of such scatter at wavelengths longer than $2,000 \text{ \AA}$ may simply be fortuitous and due to the much smaller number of observations available. The most difficult region of observation is at $\lambda > 2,000 \text{ \AA}$ where the effects of zodiacal light and starlight contamination become severe.

Below $1,000 \text{ \AA}$ observations become extremely difficult both because of the insensitivity of presently available EUV instrumentation³⁶ and the large interstellar H I absorption cross-section below $912 \text{ \AA}$ (ref. 37). A large gap in our knowledge of the UV background between 300 and $1,000 \text{ \AA}$ is due to these difficulties and more local interplanetary line emission phenomena dominate the EUV at present. After a drop of ~ 3 orders of magnitude, the background due to hot ($T > 10^5 \text{ K}$) gas in the galaxy as attenuated by intervening H I, perhaps the same gas responsible for the O VI absorption feature in hot stars³⁸, is probably the dominant diffuse emission mechanism in the EUV.

Diffuse galactic light

Interstellar dust particles may scatter starlight that was initially travelling out of the Galaxy back to an observer in the plane. The intensity of this reflected radiation at a specific wavelength λ will depend on the albedo, $a(\lambda)$, the phase function asymmetry factor, $g(\lambda)$, the optical depth of the dust and the galactic disk, and the source function of starlight. Davidson *et al.*, using then standard dust models, estimated the brightness of this source at the poles to be $\approx 250 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1}$ at $1,400 \text{ \AA}$, quite a large amount if the expected extragalactic contribution is much fainter. A convenient solution to this problem was suggested by Sandage's observations of $E(B - V) = 0$ for $b > 50^\circ$ (ref. 39). There is now considerable doubt about the validity of this result. Sandage⁴⁰ was, in fact, the first to discover faint filamentary structured reflection nebulae in the visible at latitudes as high as $|b| \approx 50^\circ$. The brightest of these filaments have an optical extinction as high as $A_v \sim 0.3 \text{ mag}$ and are clearly visible on the Sky Survey Palomar prints where they can be traced to regions not covered by Sandage's work. These clouds possibly cover a large part of the sky around the galactic poles. Recent measurements of polarization and reddening of high latitude stars⁴¹⁻⁴³ give further evidence of the existence of dust near the galactic poles.

An H I column density of at least $2 \times 10^{20} \text{ cm}^{-2}$ at the poles⁴⁴, and the canonical dust-to-gas ratio⁴⁵⁻⁴⁷, recently extended to the poles by Knude⁴³ ensure that $E(B - V) > 0.03$, and it seems safe to claim that $0.03 < E(B - V) < 0.3$ for $|b| > 50^\circ$. This result implies that dust could be a source of diffuse galactic radiation, but its presence alone is not a sufficient condition for this effect to be noticeable as the scattered intensity is also very sensitive to the values of $a(\lambda)$ and $g(\lambda)$. As far as the albedo of the dust is concerned, there seems to be general acceptance of values near $a \approx 0.5$ for $\lambda > 1,400 \text{ \AA}$, which is consistent with a recent model of the interstellar grains^{48,49}. The value of the parameter g , is, however, a subject of some controversy. Henry *et al.*⁵⁰ argued for values of g higher than 0.9, implying that the interstellar dust is strongly forward scattering, whereas others⁵¹⁻⁵³ argued for much lower values, that is, $g \approx 0.2$, or much more isotropically scattering dust grains. The above dust model predicts an intermediate value of $g \approx 0.6$ at UV wavelengths which agrees well with previously²⁶ derived value on the basis of a comparison with Jura's predictions⁵³ and with recent TD-1 observations of UV radiation around $\lambda \text{ Ori}$ (ref. 54).

The patchiness of the background radiation at $1,450 \text{ \AA}$ and the reported correlation^{26,28} with hydrogen column density does indeed suggest that backscattered galactic disk starlight is an important contributor to the diffuse background at UV wavelengths. The question then arises of whether the general level of the measured background is compatible with this view. This problem has been discussed by Jura^{53,55}.

An uncertainty in calculating the amount of backscattered starlight lies in estimating the intensity of the radiation incident on the high latitude dust. As the reflecting material is essentially seeing the Milky Way from well above the galactic plane, this problem is connected with the general question of the amount of UV radiation emerging from spiral galaxies. The curve 'Reflection nebulae' in Fig. 1 gives the predicted UV surface brightness of the densest of Sandage's filaments placed at $b \sim 90^\circ$ as a function of wavelength, calculated in terms of Jura's reflection nebula model⁵⁵ and assuming the White⁴⁹ model for interstellar dust. The incident radiation was determined by using a value of $A_v \sim 0.3 \text{ mag}$ for the optical depth of the galactic disk and by assuming that the Milky Way as seen from outside the galactic plane has the same UV spectrum as the spiral galaxies observed with the OAO 2 satellite⁵⁶. The magnitude of the UV radiation field was determined by normalizing to the total local radiation density at $1,450 \text{ \AA}$ observed onboard Apollo-Soyuz²⁶.

In spite of all the uncertainties, the general level of the theoretically expected backscattered light is in rough accord with the observed background level throughout the far UV into the visible. Note that the calculation refers to the very brightest filaments observed, and that Sandage reported filamentary

structure on scales from arc minutes to several degrees. Because most measurements are performed with instruments having fields of view larger than this, the observed smeared out background could be lower in those cases. However, the order of magnitude agreement between predictions and observations supports the claim that backscattered starlight is an important source of background light and an obstacle that must be overcome before the true extragalactic component of the UV background can be determined.

The possibility of large amounts of reflecting material existing at high galactic latitudes could limit the performance of the Space Telescope at short wavelengths but the patchiness of the filaments as indicated by Sandage and the Apollo-Soyuz data, suggests we may find rifts in the interstellar cloud cover through which it may be possible to catch unobstructed glimpses of extragalactic sources in the UV.

Extragalactic sources

The background light due to distant galaxies and quasars arises as the emission from all objects along a given line of sight looking out into the Universe. But because of redshift and lookback-time effects, more distant sources contribute to the background at a given wavelength today with light emitted at earlier epochs at shorter wavelengths. In a Friedman Universe with Hubble constant H_0 and density parameter Ω , the background intensity I_{λ_0} at wavelength λ_0 due to a class of objects with luminosity density ϵ_{λ_0} can be written⁵⁷

$$I_{\lambda_0} = \left(\frac{c}{H_0} \right) \frac{\epsilon_{\lambda_0}}{4\pi} A_{\lambda_0}$$

with A_{λ_0} , 'the accumulation factor' given by

$$A_{\lambda_0} = \int_0^\infty \chi_\lambda(z, \lambda_0/(1+z)) \phi(z) (1+z)^{-4} (1+\Omega z)^{-1/2} dz$$

$\chi_\lambda(z, \lambda)$ describes the mean spectra and $\phi(z)$ the comoving density evolution of the objects under consideration, both normalized to $\chi_\lambda(0, \lambda_0) = 1$ and $\phi(0) = 1$.

To estimate the expected UV background due to galaxies and QSOs, detailed information on the UV spectra and evolution of these objects is required. Conversely, measurements of the background radiation field can, in principle, constrain different galaxy and quasar evolution hypotheses.

Despite recent advances in space borne instrumentation, surprisingly little is known about the UV spectra of galaxies. This is mainly because the instruments that have been launched up until now have been of moderate size and optimized for stellar work. For example, IUE galaxy observations have been restricted to spectra of the central regions of elliptical galaxies and bulge components of spirals, that is of portions of objects with large surface brightness. The spectra of these regions fall off rapidly shortwards of the B -band at $\sim 4,400 \text{ \AA}$ down to $\sim 2,000 \text{ \AA}$ after which a relatively flat residual remains at a level of $f_\lambda \sim 0.05 f_\lambda(B)$ (refs 58, 59). The origin of the far UV galactic radiation is controversial but if this behaviour is assumed typical of all galaxies, the expected galaxy background contribution, neglecting evolution and calculated for $\Omega = 0$, is plotted as the curve 'Galaxies/1' in Fig. 1. Note that there is an overall uncertainty in all calculations of this type of a factor ~ 2 due to the uncertainty in the total B -luminosity density in galaxies alone, but that the calculations are otherwise insensitive to the cosmological model.

In the other extreme case one can, following Anderson *et al.*²², adopt as the typical galaxy spectrum the mean Scd galaxy spectrum⁵⁶ observed with OAO 2, extrapolated to the Lyman limit. The expected background contribution in this case, neglecting evolution, is shown as the curve 'Galaxies/2' in Fig. 1.

Thus if all galaxies have as energetic short wavelength spectra as the Scd galaxies observed by the OAO 2, and our extrapolation to the Lyman limit is valid, the integrated light from distant galaxies could be a substantial contributor to the UV background light.

Because all galaxies are not spirals, the true galaxy background is probably between the two cases shown in Fig. 1. Recent estimates of the colour distribution of galaxies^{60,61}, suggest that only about 5–10% of the total B -light from galaxies is emitted by ellipticals, implying that the latter estimate is perhaps the most realistic. Tinsley⁶² showed that the accumulated galaxy radiation at visible wavelengths is very sensitive to the effects of galaxy evolution, but the situation in the far UV may be less complicated⁶³. This is because the short wavelength light emitted by spiral galaxies originates almost entirely from the young, short-lived O and B star population continuously forming in the spiral arms. Aside from the disturbing effects of dust absorption, the intensity and slope of the far UV spectrum of a spiral galaxy at a given epoch directly mirror the rate of formation and the initial mass function of the young hot star population. If, for example, the initial mass function is independent of epoch and the rate of star formation decreases exponentially with the galaxy age, this will give rise to an exponential increase in the overall far-UV luminosity of spiral galaxies with look back time⁶⁴. In this way, the background measurements shortward of $2,000 \text{ \AA}$ together with the adopted OAO 2 spiral spectrum set a rough lower limit on the e-folding time for stellar formation of about a quarter of the Hubble time or $\sim 5,000 \text{ Myr}$. Taking such evolution effects into account will raise our background estimate $\sim 50\%$ shortwards of $2,000 \text{ \AA}$, making galaxies even more important contributors to the UV background.

Results from the HEAO 2 satellite concerning the contribution to the X-ray background from quasars⁶⁵, suggest that these objects might be expected to also play an important part in generating the background at UV wavelengths. Adopting the estimated QSO-luminosity function⁶⁶ and $\phi(z) = e^{10z/(1+z)}$ density evolution⁶⁷ together with a $\chi_\lambda \propto \lambda^{-1}$ powerlaw spectrum extending unattenuated beyond the Lyman limit, the expected background is shown as the curve 'Quasars' in Fig. 1. A quasar turn on epoch of $z \sim 3$ was assumed, but with the adopted density evolution, the result is not very sensitive to the exact redshift at which QSOs first appear. Although the predicted background falls far below the UV measurements, it is interesting that the integrated light from quasars does exceed the EUV background in Fig. 1. Because this radiation is reasonably well accounted for by hot interstellar gas emission³⁴, this suggests that QSOs are less bright at soft X-ray energies than expected from an extrapolation of the $\chi_\lambda \propto \lambda^{-1}$ continuum observed in the optical, either because of intrinsic shielding or because of a true steepening of the continuum⁶⁸.

Although the integrated light from quasars seems unimportant in connection with the UV background, a large fraction of the QSO radiation is emitted at wavelengths shortwards of the Lyman limit at distant epochs and may play an important part in photoionizing the mythical intergalactic medium.

Although most cosmological information suggests that the Universe is open by a large margin^{69,70} and that the major fraction of matter in the Universe is in the form of galaxies, many attempts have been made to detect a dense intergalactic medium (IGM).

Whereas the observed X-ray background could arise as emission from a very hot ($T = 10^8 \text{ K}$) IGM^{71,72}, the intensity of the background at UV wavelengths is relevant in the case of a "lukewarm" or photoionized IGM with a temperature between $\sim 10^3 \text{ K}$ and $\sim 10^5 \text{ K}$. Several IGM models which predict temperatures in this range, at least at some epoch, have been proposed^{73–77}. This case has been discussed recently in terms of the resulting background radiation^{26,57}.

At temperatures $10^3 < T < 10^5 \text{ K}$ a mixture of hydrogen and 10% helium radiates strongly in the two resonance lines of H I at $1,216 \text{ \AA}$ and He II at $304 \text{ \AA}$. This line emission, smeared by the redshift, should turn up as background radiation in the UV. The two more or less complementary mechanisms for generating such line radiation are (1) collisional excitation which dominates in an IGM heated by dissipative processes; and (2) recombination which dominates in a highly photoionized medium.

Although the emissivity in the collisional excitation case is in general much larger than in the recombination case, very specific conditions, that is a combination of incomplete ionization and high electron temperatures, are necessary to maintain this large emissivity and corresponding high level of background radiation.

Very strict upper limits on the possible amounts of dense, well-distributed intergalactic neutral hydrogen are set by the lack of strong absorption troughs between emitted and observer frame Ly α in the spectrum of high redshift quasars⁷⁸. It follows that if a dense IGM does exist, the intergalactic hydrogen must be highly ionized, typically to a level of 1 p.p.m. This severely constrains all IGM models and reduces the chances of detecting the IGM in emission by excluding the case for a $T \approx 2 \times 10^4$ K IGM radiating strongly in Ly α by collisional excitation.

The curve 'Ly α ' in Fig. 1 shows the expected background contribution due to redshifted recombination Ly α from a dense, fully ionized IGM of temperature 10^4 K, having a closure density appropriate for a Hubble constant of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The expected background intensity increases at longer wavelengths corresponding to emission from earlier, more dense, epochs as the recombination emissivity is proportional to density squared. This estimate scales with cosmological parameters as $\Omega_b^2 h_{50}^3$, where Ω_b denotes the IGM density in units of the critical density and h_{50} the Hubble constant in units of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

Regarding redshifted He II 304 Å line radiation, there is no direct information on the ionization of intergalactic helium, but the Space Telescope will make it possible to carry out the He I 584 Å and He II 304 Å equivalents of the Gunn–Peterson test⁶⁸, and set upper limits on the concentrations of neutral and once ionized intergalactic helium. Until then it cannot be completely excluded that the IGM at some epoch has gone through a phase of intense collisional excitation generated He II 304 Å emission as predicted by some IGM-reheating models^{74,76}. Although the reported 1,450 Å background feature^{21,32} is suggestive of such radiation, its existence is in doubt. In any case, at 1,450 Å the observations^{22,25,26} can be used to set a rigorous upper limit to an extragalactic component of $300 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ Å}^{-1}$.

The curve 'He II 304' in Fig. 1 shows the expected background due to redshifted He II 304 Å recombination radiation from a fully ionized IGM calculated for a temperature of 10^4 K and the combination $\Omega_b^2 h_{50}^3 = 1$. The He II 304 Å emissivity in the collisional excitation case with these parameters is, at most, a factor ≈ 5 higher than that of the recombination case, still giving a background well below the measurements.

The measured intensities of the UV background do not set very interesting limits on the density of a photoionized intergalactic medium in terms of redshifted IGM recombination emission ($\Omega_b^2 h_{50}^3 \leq 280$ from Ly α and $\Omega_b^2 h_{50}^3 \leq 70$ from He II 304 Å). A more interesting constraint is that, as any existing IGM must be highly transparent according to the Gunn–Peterson test, the ionizing flux that maintains the implied high level of ionization must turn up as background radiation at the appropriate wavelengths. The curve 'Photoionized IGM' in Fig. 1 shows the background level produced by the redshifted Lyman continuum radiation necessary to photoionize a dense intergalactic medium to the required level. This curve also scales with cosmological parameters as $\Omega_b^2 h_{50}^3$, so even assuming that the lowest background measurement at 1,450 Å of $300 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ Å}^{-1}$ consists of the sum of contributions from redshifted recombination Ly α from $z = 0.2$, redshifted Lyman continuum radiation from $z = 0.6$ and redshifted recombination He II 304 Å radiation from $z = 3.8$ leads to the inequality $\Omega_b^2 h_{50}^3 \leq 10$, leaving a wide margin for a closed universe in the dense photoionized IGM picture. We cannot rigorously exclude that a major portion of the UV background radiation is redshifted Lyman continuum radiation, but this interpretation seems unlikely because no known sources, quasars included, seem capable of producing such a large ionizing flux.

The discussion above refers to a dense well-distributed intergalactic medium. On the other hand a very plausible interpretation of the numerous absorption lines seen shortwards of

emitted Ly α in the spectra of high redshift quasars is that at least some of these lines arise as Ly α absorption in intervening intergalactic clouds^{79–81}.

Taking clumping into account increases the IGM emissivity by the 'clumping factor', $C(z) = \langle n^2 \rangle / \langle n \rangle^2$, but the redshifted line radiation decreases in intensity towards longer wavelengths because the clumping is expected to increase with time at least as $C(z) \propto (1+z)^{-3} \propto \lambda^{-3}$, corresponding to comoving clouds of fixed dimensions²⁶. As an example, curves 'Ly α /C' and 'He II 304/C' show the expected redshifted Ly α and He II 304 Å background from unevolving photoionized intergalactic clouds, for simplicity calculated for $\Omega_b^2 h_{50}^3 C(0) = 1$. Sargent *et al.*⁸¹ presented a homogeneous sample of QSO Ly α absorption lines observed in high resolution spectra of six quasars. Unfortunately, the interpretation of these spectra in terms of the physical parameters of the absorbing matter is not unique. However, Sargent *et al.*⁸¹ proposed a low density IGM photoionized by quasars consisting of clouds of density $\sim 10^{-4} \text{ cm}^{-3}$ and temperature $T \sim 3 \times 10^4$ K embedded in a medium with a density $\sim 10^{-5} \text{ cm}^{-3}$ and temperature $\sim 3 \times 10^5$ K (at redshift $z \sim 2.5$). If so, the expected background due to IGM emission is very low, as these parameters translate to $\Omega_b^2 h_{50}^3 C(0) \approx 10^{-1}$ for the intergalactic clouds giving rise to the QSO absorption lines and $\Omega_b^2 h_{50}^3 \approx 10^{-2}$ for the surrounding medium. As far as the background radiation is concerned, the above is mainly interesting in that the true integrated light from quasars must be similar to that given in Fig. 1 for these objects to supply sufficient Lyman continuum radiation to photoionize the intergalactic clouds to the required level.

The expected level of IGM emission under reasonable assumptions is far below the measured UV background intensity. The present measurements do not rule out a dense photoionized IGM, but future observations with higher sensitivity together with a better understanding of the other background contributions should place interesting limits on this possibility.

The interstellar radiation density

UV photons have sufficient energy to ionize, dissociate and heat the interstellar medium^{82–84}. The heating effect of UV photons on diffuse clouds and the periphery of larger clouds is quite important. For example, the proposed heating mechanisms of diffuse interstellar clouds require UV photons to photoeject electrons from grains^{85,86} or to excite H₂ to vibrationally bound levels⁸⁷. Absorption of UV radiation by H₂ controls the abundance of this and other molecules in cool clouds⁸⁸.

Most of the UV radiation in the solar neighbourhood probably originates in O and B stars^{88–90} and hot white dwarfs¹⁴ within 1 kpc of the Sun. Just below the H I ionization threshold at 912 Å, the mean free path of UV photons is small³⁷ and the radiation field is characteristic of a smaller region around the

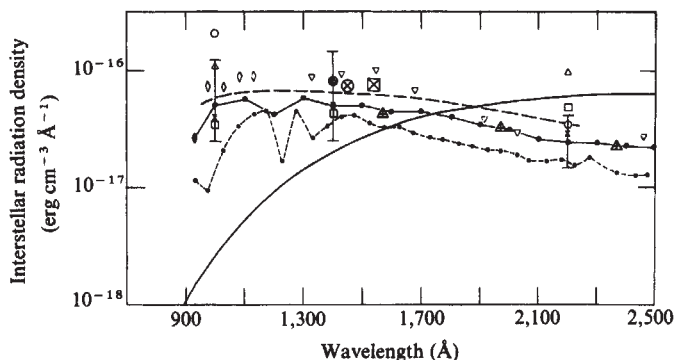


Fig. 2 Observations and theoretical estimates of the interstellar radiation density as a function of wavelength. Sources of the observations: $\square$, ref. 17; $\otimes$, ref. 26; Δ , ref. 97. Sources of the calculations: $\square$, ref. 98; $\bullet$ — $\bullet$, ref. 99; —, ref. 89; $\times$, ref. 96; — — —, ref. 11; $\diamond$, ref. 88; $\circ$, ref. 100; ∇ , ref. 101; Δ , ref. 102; $\bullet$ — $\bullet$, ref. 97.

Sun out to ≈ 10 pc or so. The field in this case will be dominated by the combined effects of the ≈ 10 early type B stars to be found at these distances from the Sun⁹¹ and hot white dwarfs, subdwarfs, novae, and other possible EUV sources⁹²⁻⁹⁴. Enough radiation below 912 Å will seriously affect the He I/H I ratio in the ISM and observations of the interstellar/interplanetary wind^{89,95}.

Several theoretical or empirical determinations of the expected energy density in the UV have been attempted. For heating, ionization and dissociation of ISM atoms and molecules, the important parameter is clearly the mean intensity of the UV radiation field or the equivalent energy density U_λ . The results of the calculations of U_λ in $\text{erg cm}^{-3} \text{Å}^{-1}$ as a function of wavelength are plotted in Fig. 2.

The computations shown in Fig. 2 are hampered by many of the uncertainties encountered when establishing the total luminosity of the galaxy because they depend on an integration of all the possible galactic sources of UV radiation. Specifically, the uncertainties reflect our incomplete knowledge of the flux and space density of hot stars, especially below 1,200 Å, and of the distribution and scattering characteristics of dust grains. The calculations are, in principle, very sensitive to the exact location in space but as long as the chosen spot is well removed from the vicinity of very hot stars and associations, this effect is not very important in practice⁹⁶.

Only three direct observations of the interstellar radiation density exist: Henry *et al.*'s rocket observation¹⁷, the ASTP results²⁶, and the TD-1 sky survey⁹⁷ indicated in Fig. 2. The measurements are compatible with Witt and Johnson's, Henry's and an extrapolation of Jura's results but are higher than the Gondhalekar and Wilson, Grewing and Dunham estimates. Grewing's curve can be made compatible with the observations if $n_e \approx 0.2-0.4 \text{ cm}^{-3}$, a value much higher than the canonical value of 0.03 cm^{-3} from pulsar dispersion measures, but not to be excluded *a priori*.

Conclusions

The scatter of the data and of the theoretical expectations in Figs 1 and 2 are due, probably in roughly equal measure, to the natural variation of the physical parameters and to observational and theoretical difficulties. This is not unexpected if the present observations are dominated by the dust scattering mechanism described above. Thus future work should explore systematically the magnitude and extent of the galactic dust source by an all-sky survey at high sensitivity (a 3σ detection of $\approx 100 \text{ photons cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}^{-1}$) and moderate angular ($0.1-0.5^\circ$) and spectral resolution ($10-100 \text{ Å}$). A direct comparison with optical and far IR deep surveys as described by Sandage⁴⁰, King *et al.*¹⁰³ and Drapatz¹⁰⁴ would be very useful in this context. Such surveys in the UV are being planned for some large ($\approx 1\text{-m}$ diameter) space borne telescopes and by the wide field far UV camera on Spacelab I¹⁰⁵.

The background spectrum at moderate spectral resolutions ($\sim 10 \text{ Å}$) can provide valuable information on the emission mechanism. Measurement of the polarization of the radiation field would be valuable. Jura⁵⁵ has shown that one can expect a linear polarization of the backscattered light by dust grains up to 11% at $b = 60^\circ$, an amount easily detectable by a simple UV polarimeter mounted at the focus of presently available or planned UV telescopes. Such a measurement would be an important test of hypotheses on the origin of the observed diffuse UV background. However, extragalactic radiation might also be polarized due to transmission through aligned dust grains.

A better understanding of or a bigger effort at minimizing or avoiding contaminating sources of diffuse emissions is clearly required. Spacecraft orbits should be high enough to avoid most of the terrestrial molecular band emission in the UV that is at least above $\approx 500 \text{ km}$ but not in the radiation belts where particle induced background can be severe. Zones subject to auroral precipitation should be avoided completely. A completely opaque shutter periodically occulting the outside

world and precise calibrations of overall throughput and field of view are a must for accurate absolute measurements of UV intensities. Techniques by which Ly α is rejected by at least a factor of 10^6 must be perfected for the region 1,050–1216 Å where no crystal filter or grating on its own can do the job.

As long as the field of view is small and/or the observations are made near the galactic poles, point sources can be avoided or corrected for fairly accurately at least in the 1,200–2,000 Å range especially if deep Schmidt plates of the UV targets are available. Below 1,200 Å more information on the space density and the luminosity of UV emitters is badly needed. A complete survey of the sky in the 100–1200 Å band of the spectrum down to limiting sensitivities of $M_v \approx 15-20$ is also critical. A UV survey of this type is being planned by NASA for an Explorer mission.

Present observations summarized in Fig. 1 set very weak constraints on extragalactic sources of UV emissions. The question remains about the general validity over the sky of the observational lower limit of $\approx 300 \text{ photons cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{Å}$ at 1,450 Å that presently pertains only to the limited region surveyed so far. We also need to establish whether the observed minimum is due to a veil of backscattering dust or to an extragalactic source observed through a rift in the galactic dust cloud. This will be a difficult point to resolve and polarization measurements and additional studies of dust to gas correlations may be required.

Additional sources of diffuse UV emissions might be envisioned. For example, recent observations from IUE¹⁰⁶ have been interpreted as evidence for the existence of a hot gaseous halo around our galaxy. This hot gas cools by emitting lines in the UV which could turn up in or influence moderate to high spectral resolution observations of the far UV background.

Clearly a better understanding of all contributions to the UV background is necessary before any information of cosmological significance can be distilled from the observations. First, integrated galaxy and QSO spectra extending to very short wavelengths are required to assess the background contributions and the output of ionizing radiation from these sources. With the Space Telescope it should become possible to subtract the bulk of the galaxy and QSO background light in specific fields in much the same way as star subtraction is now performed, leaving a residual presumably due to IGM emission. The intensity of this residual radiation together with information on the physical state of intergalactic Helium obtained from the He I 584 Å and He II 304 Å equivalents of the Gunn-Peterson test should set interesting constraints on or establish the amount of intergalactic matter present in the Universe.

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Note added in proof: Very recently, various particle experiments have indicated that neutrinos may possess mass. Because large numbers of light leptons are produced in the big bang, the implications of this for cosmology are far reaching¹⁰⁷. One possibility is that such massive neutrinos are unstable to radiative decay. An extragalactic component of the UV background could conceivably be due to the redshift-smeared emission from such a cosmological sea of decaying neutrinos¹⁰⁸.

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ARTICLES

Anomalous region on Mars: implications for near-surface liquid water

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An anomalous region has been identified on Mars from the 1971 and 1973 Earth-based Goldstone radar data. This region is characterized by coincident very high radar reflectivities and unusual smoothness. Very few realistic surface morphologies can generate such return-signals. Liquid water within ~50–100 cm of the surface is one possibility, an interpretation strengthened by an apparent seasonal variation in surface reflectivity for the same locality.

ANALYSIS of radar measurements of the martian surface provides the possibility of interpreting textural/compositional differences of materials on a scale unobtainable from orbital images^{1–3}. Estimations of the dielectric constant (from radar reflectivity) and small-scale slope statistics (see, for example, the C-factor analysis developed by Hagfors⁴) can be used to help characterize surface units⁵ or to delineate areas with unusual material properties. Radar can provide additional evidence regarding the existence of near-equatorial regions of H₂O outgassing ('oases') as proposed by Huguenin *et al.*^{6,7}. Liquid water should produce an obvious effect on the radar characteristics of the surface (if the overburden is relatively transparent to the

radar energy), because of the very high dielectric constant of liquid water (~10 times that of solid rock and 20 times that of water ice).

Data base

Earth-based radar measurements of the martian surface were carried out at the Jet Propulsion Laboratory's Goldstone Facility by Downs and others² in 1971 and 1973. The combined set of observations gave almost complete coverage between latitudes 14 and 22° S. Where possible, the measurements were reduced to obtain inherent reflectivity and smoothness (C-factor) of the surface material, as well as topography and other data not

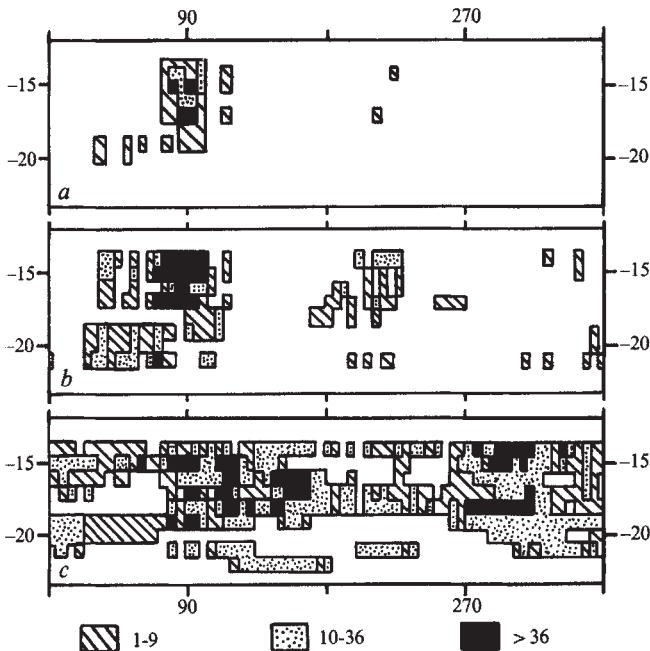


Fig. 1 Number density of radar sample points with given characteristics per 1° lat., 5° long. sample bins. *a*, Reflectivity $> 9\%$ and *C*-factor $> 3,200$ coincidentally; *b*, *C*-factor $> 3,200$; *c*, reflectivity $> 9\%$. Note that although data points with high reflectivity or *C*-factor occur over a wide area of Mars, coincident high values in both parameters are found exclusively at the 'oasis' areas proposed by Huguenin *et al.*^{6,7} (Solis Lacus, at 20°S , $80^\circ\text{--}90^\circ\text{W}$; and Noachis-Hellespontus, at $15^\circ\text{--}25^\circ\text{S}$, $305^\circ\text{--}315^\circ\text{W}$).

considered here. In some cases the *C*-factor and hence the reflectivity could not be obtained, usually because of low signal strength resulting from rough and low-dielectric constant surfaces. In any case, a very low smoothness (*C*-factor) measurement would be suspect because of the limited Doppler bandwidth of the radar, usually resulting in an overestimate of the smoothness and a consequent underestimate of the reflectivity.

Hence the most productive type of search seemed to be for a uniform layer of liquid or of damp soil, where both reflectivity and *C*-factor were expected to be high.

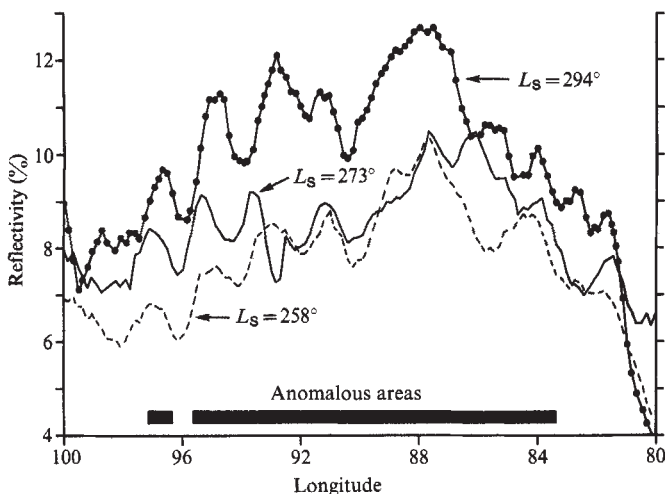


Fig. 2 Temporal variations in radar reflectivity for an area of Solis Lacus at $15.43\text{--}15.81^\circ\text{S}$. Curves represent a running five-point average at 0.16 long. intervals. Radar data was taken during 1971 ($L_s = 258^\circ$) and 1973 ($L_s = 273^\circ$ and 294°) Mars oppositions at latitudes 15.44 , 15.43 and 15.81°S respectively. The 'anomalous areas' refer to parts of the ground track at $L_s = 294^\circ$ where reflectivity is $> 9\%$ and *C*-factor $> 3,200$. See also Fig. 4a.

Analysis

The data were scanned for regions with a coincident high reflectivity and smoothness. Limits of 9% reflectivity and $3,200$ *C*-factor (r.m.s. slopes $< 1^\circ$) were chosen somewhat arbitrarily. Variations from 9 to 11% and from $2,400$ to $3,200$ respectively had only a slight effect on the shape of the anomalous area and on the number of observation points. For these measurement values, the formal errors (standard deviations) were $\sim 10\%$ of the data value.

Figure 1a illustrates the areocentric position and number densities of these high-reflectivity, high-smoothness measurements. Only two regions on the planet share this combined radar characteristic. The most prominent is a region centred at 16°S , 90°W . There is also a pair of smaller areas to the north-west of Hellas. These two regions are remarkably close to the Solis Lacus (25°S , 85°W) and Noachis-Hellespontus (20°S , 310°W) oases proposed by Huguenin *et al.*^{6,7}

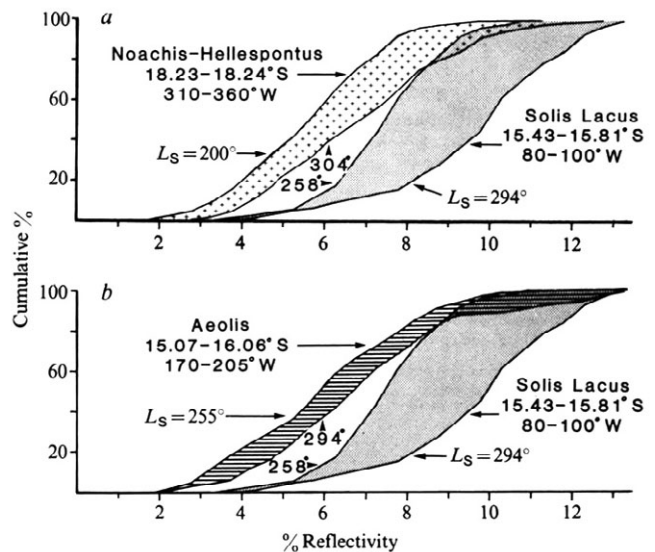


Fig. 3 The seasonal variations in radar reflectivity are compared here for three regions of Mars. The horizontal axis is reflectivity and the vertical axis is the percentage of data points having less than the indicated reflectivity. *a*, Compares the strongly anomalous Solis Lacus area with the weakly anomalous Noachis-Hellespontus region. *b*, Compares Solis Lacus with an area in Aeolis, which is taken to be representative of Mars as a whole and shows no anomaly. Note that all three areas display an increase in percentage reflectivity with increasing martian season, but that Solis Lacus has a much more pronounced seasonal change, in addition to its higher absolute radar reflectivity.

For comparison, Fig. 1b, c indicates the areas having only their reflectivity or *C*-factor above the defined limits. Note that areas of high reflectivity are abundant, indicating a profusion of well-compacted or rocky soils on the martian surface. Areas of high smoothness are clearly less common, although they are not only restricted to the anomalies described here.

As an argument for the reality of the anomalous areas, note that the map of Fig. 1a includes radar measurements from both the 1971 and 1973 conjunctions. Almost no anomalous data points occur elsewhere on the planet, despite the near-global coverage of the data set between 14 and 22°S . In the 300×300 km anomaly itself, on the other hand, there are several hundred points.

Seasonal variability

Additional characteristics of Solis Lacus can be determined by comparing radar data derived for nearly coincident ground tracks at different times of the martian year. Note that practical limitations of Earth-based radar systems dictate that radar

observations of the sub-Earth-region must be made near opposition, and hence near local martian summer. Winter measurements would at best be extremely difficult to make using Earth-based radar.

A combination of data from the 1971 and 1973 oppositions provides one example of overlapping radar swaths: Latitudes 15.45°S , 15.43°S , and 15.81°S were sampled between 80 and 100°W during the seasonal range from early to middle local martian summer ($L_s = 258^{\circ}$, 273° and 294° respectively). Note that, as L_s represents the longitude of the Sun as seen from Mars, the difference between $L_s = 258^{\circ}$ and 294° is roughly equivalent to the shift from early June to mid-July on Earth (Northern Hemisphere). Because of the nearly identical locations of these radar profiles (the north-south extent of the radar resolution cell is 1.3° , giving an overlap of at least 70% between swaths), the reflectivity curves for identical surfaces should be very similar. However, Fig. 2 indicates that there is a discrepancy in the reflectivity between $L_s = 258^{\circ}$ and 273° . The 273° profile indicates an increase of 1.0–1.5% in the reflectivity compared with the same longitude at $L_s = 258^{\circ}$, while the 294° profile shows a further enhancement of 2.5–3.5%. For large parts of the region between 82 and 100°W , there is a systematically higher reflectivity with advancing L_s , that is, with season. Such a phenomenon seems to be real, as some parts of the data (for example, between 83 – 84°W and 88 – 92°W for $L_s = 258^{\circ}$ and 273°) show no difference in reflectivity despite their respective acquisition dates in 1971 and 1973. This is strong evidence for the adequacy of the calibration of the original radar data, and

also for the lack of any temporal variation in the data from instrumental causes.

In another test for possible systematic effects, we are searching the radar data at areas other than the observed anomaly for quasi-seasonal variability. Some of the results are depicted in Fig. 3. Figure 3a shows a plot of the range of variability of the reflectivity with L_s for Noachis-Hellespontus, which contains the only cluster of anomalous points outside the Solis Lacus region. Figure 3b shows Aeolis as an example of a non-anomalous region. Figure 3 demonstrates the 'seasonal' variability in much of the observed area, in the same sense (increased reflectivity with advancing summer), but Solis Lacus exhibits the greatest range by more than a factor of 2 of those areas so far examined. The general variability may indicate scattered expressions of near-surface water and/or permafrost, or possibly a low-level, systematic and time-dependent flaw in the calibration.

Discussion

Measurements showing a high radar reflectivity (high dielectric constant) would be expected not only for water surfaces, but also for large areas of smooth rock within 1 m of the surface. In these conditions, anorthositic rock, with a dielectric constant K of ~ 5 , would have a reflectivity of about 15% (basalt, with $K = 9$, is $> 25\%$; water, with $K = 81$, is $> 65\%$) if it covered the entire surface area of the radar cell ($\sim 750\text{ km}^2$). Figure 4 presents Viking Orbiter images of the same area as Fig. 2. Although few craters larger than $\sim 10\text{ km}$ diameter are within the radar

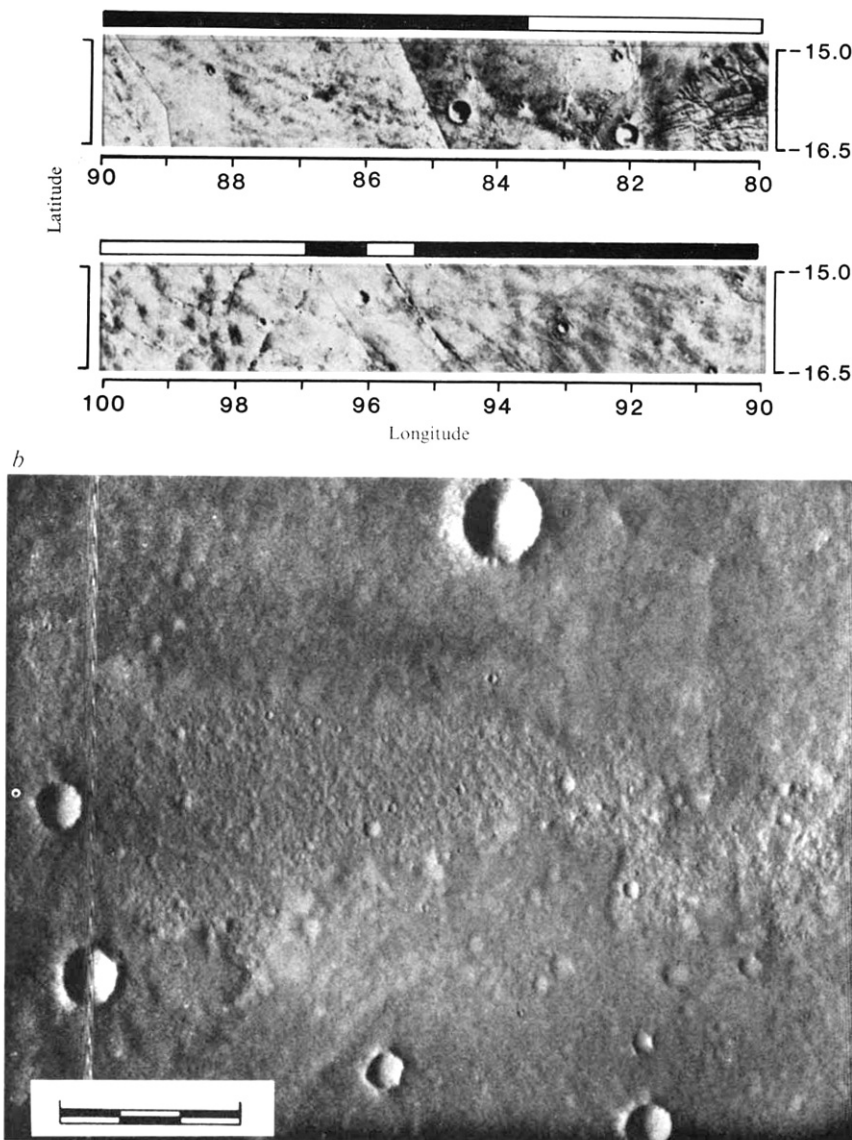


Fig. 4 Viking Orbiter Images of the anomalous radar areas. *a*, Ground track shown in Fig. 2. *b*, High resolution for an area in Solis Lacus at 18.7°S , 91.4°W ; C -factor, 3,186; $L_s = 250$; reflection, 10.77%. Note that the images do not support a hypothetical smooth, mantle-free surface as an explanation of the radar anomaly. Numerous 10-km craters and lava flow fronts are in evidence. (USGS photomosaics I-1183 and I-1190; and Viking frame 773A10.) Scale bar, 3 km.

ground track, many moderately well preserved lava flow fronts can be easily identified. Morphologically comparable lava flows elsewhere on Mars either have radar reflectivities of 2–6% or *C*-factors <2,000 (refs 5, 8, 9). Unless the small-scale surface texture of the Solis Lacus region is unrelated to its large-scale appearance in Fig. 3, the hypothesis of an extensive rock pediment is untenable as an explanation for the high values of radar reflectivity and *C*-factor.

Moreover, to explain a seasonal variability in the reflectivity of a solid rock surface, one must hypothesize, for example, an absorptive overburden which is regularly scoured away as the summer progresses. The thickness of overburden required for the observed changes in dielectric constant have been calculated on the basis of published dielectric constants and absorption lengths for basaltic rock and loosely-compacted rock powder¹⁰. Although resonant effects of thin highly-planar soil and rock layers might be observed in laboratory conditions, such conditions do not exist on a planetary surface. Consequently, the reflectivity was calculated for a random-thickness soil layer masking a solid rock surface. Assuming dielectric constants of 2.0 for soil and 8.0 for rock, the net reflection coefficient for bare rock is 23%; for a soil layer at least 14 cm (one wavelength) thick, is 11.1%; and drops to 6% for a soil depth of 1.4 m. For lunar rock chemistry rather than terrestrial rock chemistry, the 6% reflectivity would require >10 m of rock-free soil. The problem is complicated by other factors such as variation in soil porosity with depth, but the conclusion, nevertheless, is that for rock and soil surface to exhibit a measured reflectivity of more than 11%, the soil thickness must be in the range 10–20 cm over the entire area; and that an additional deposit of the order of 1 m of soil would be required to reduce further the dielectric constant to 8%. Although there is undoubtedly a seasonal redistribution of martian dust, photographs at the Viking Lander sites¹¹ do not show the buildup of the tens of centimetres of dust that would be needed to effect a change from 11 to 8% in

the average radar reflectivity. Alternative explanations, such as seasonal changes in the bulk chemistry of the near-surface rocks, also seem to be unlikely.

Conversely, a transition from frozen water (reflectivity >7%) to liquid water (reflectivity >64%) would produce just such a striking difference in the observed radar reflectivity. Surface temperatures in the range 230–250 K have been observed, for example, at the Viking Lander sites¹². While this is well below the freezing temperature for pure water, salt solutions may well exist, and have been shown¹³ to be stable at temperatures as low as 210 K.

We conclude, therefore, that only surface materials containing liquid water are consistent with: (1) the coincident very high radar reflectivities and *C*-factor values; (2) the photographically observed surface morphology; and (3) the increase in reflectivity values from spring to summer. Some inferences can also be made about the regional extent of the near-surface expression of the liquid water at Solis Lacus. An extensive liquid water surface would have a much higher reflectivity than the observed maximum value of ~16%. More likely is a reduction in the reflectivity by an overlying layer of soil, as outlined above for the rock–soil combination, or, alternatively (or additionally), the liquid water might be observable only through random patches of the surface because of variations in the thickness or density of the overburden. In the latter case, the observed reflection values would be from a mixture of high and low reflectivity surface elements. In this case, the *C*-factor measurements would depend mainly on the highly reflecting regions (the oases) rather than the dry intervening areas. In either case, we are convinced by the radar data that liquid water does indeed exist near the surface of Solis Lacus during southern hemisphere summer.

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Highly structured water network in crystals of a deoxydinucleoside–drug complex

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X-ray diffraction analysis of crystals of the intercalative complex between the deoxyribonucleoside phosphate d(CpG) and the mutagen proflavine shows a highly structured arrangement of water molecules linked together by networks of hydrogen bonds to form four edge-linked pentagons per asymmetric unit. These pentagons have a general role in maximizing hydrogen bonding at 3.4-Å intervals. The conformation of the deoxyribose sugar ring at the 3' end of one strand can depend on its local aqueous environment.

THE role of water in stabilizing and mediating the structure and conformation of nucleic acids has long been the subject of study and speculation (see, for example, refs 1–3). The conformational transitions of DNA are dependent on the level of humidity; it has been established^{4–6} that in A-DNA each nucleotide is associated with about 8–10 water molecules whereas B-DNA has a rather greater number. As the primary hydration shell of a nucleotide can maximally accommodate fewer water molecules

than even this lower limit, it has been suggested^{2,7,8} that 'bulk' water molecules are involved in addition to those actually hydrogen-bonded to the bases and backbone.

Features of the environment involving these latter water molecules have been investigated in several ways; the crystal structures of both ribodinucleoside phosphates GpC⁺ and ApU¹⁰ have all potential nucleobase hydrogen-bond donors and acceptors utilizing their maximum possible water interactions;

Table 1 Characteristics of two crystals of the 2:2 complex of d(CpG)-proflavine

	Crystal 1	Crystal 2
Final unit cell	$a = 32.871 \text{ \AA}$ $b = 22.187 \text{ \AA}$ $c = 13.506 \text{ \AA}$ Volume = $9,850 \text{ \AA}^3$	$a = 32.991 \text{ \AA}$ $b = 21.995 \text{ \AA}$ $c = 13.509 \text{ \AA}$ Volume = $9,802 \text{ \AA}^3$
Resolution limit	1.00 $\text{\AA}$	0.83 $\text{\AA}$
No. of water molecules	27.5	26

this finding has been used to investigate sequence-specific nucleic acid recognition¹¹ using water molecules as a probe of a variable group. The hydration of base pairs has been examined by quantum-mechanical methods¹². Recently the solvent-accessible surfaces of tRNA^{8,13} as well as of double-stranded DNA and RNA have been investigated, the overall important conclusion being that conformationally different forms of double-helical polynucleotides exhibit differing degrees of exposure to the protons of clustered water molecules.

It remains true that there are no simple descriptions for the water structures in oligonucleotides^{14,15} which can be extrapolated to generalized descriptions for the hydration of nucleic acids.

We report here on the details of the arrangements of water molecules in crystals of the deoxydinucleoside phosphate d(CpG) intercalative complex with the mutagenic agent proflavine¹⁶. This structure reveals hitherto unsuspected highly ordered and structured networks of water molecules. These arrangements may provide insight into the structure of aqueous environments around nucleic acids in general, and also demonstrate the importance of water molecules in maintaining oligonucleotide and nucleic acid structure.

Crystallographic details

X-ray diffraction data were collected on two different crystals of the d(CpG):proflavine complex. Table 1 details some data for these crystals. The superior quality of crystal 2, coupled with more observed data compared with crystal 1, enabled the structure to be successfully and fully refined by standard full-matrix least-squares techniques. Aspects of the refinement and the conformational features of this structure have already been

discussed elsewhere¹⁶. Crystal 1 proved less amenable to conventional techniques of refinement, which initially resulted in unacceptable and abnormal bond distances and angles. Successful refinement of the structure obtained from data on crystal 1 was contingent on (1) a careful procedure of constrained restrained refinement, and (2) the realization that the 3' deoxy-ribose sugar in one strand (of clear C3'-endo conformation in crystal 2) was disordered between ~50% populations of C2'-endo and C3'-endo conformers. We shall show that this disorder phenomenon is related to the water environment. Except where otherwise stated, the following descriptions are of the crystal 2 structure.

To determine the water structure, difference Fourier maps were calculated. A peak was considered to be a water molecule only if it formed reasonable hydrogen-bonding contacts with the duplex or with another water molecule candidate. The positions and temperature factors were refined; a peak was accepted to be a water molecule if there was convergence and the temperature factors remained below $B = 30 \text{ \AA}^2$. After the refinement, detailed analysis of distances and angles involving water molecules revealed the networks to be described. Thus, no bias towards any particular arrangement was introduced during structure solution and refinement. Water-contact distances in the structure average $2.8 \text{ \AA}$, with a few deviating from this norm (Table 2). The maximum distance found was $3.2 \text{ \AA}$, which, although near the limit of an acceptable hydrogen-bonding distance, did fulfill the other requirements for hydrogen-bonding geometry. The average mean-square amplitude of vibration for the first-shell water molecules is $0.18 \text{ \AA}^2$, and for the bulk waters is $0.26 \text{ \AA}^2$; these relatively high degrees of thermal motion and the large estimated standard deviations of the positional parameters necessarily lead to some ambiguities in geometry which cannot be resolved by X-ray data alone. Unfortunately, the small crystal size does not make neutron analysis possible at this time.

Overall view of the crystal structure

The asymmetric unit of the crystal contains a 2:2 complex of d(CpG) and proflavine. These pack as infinite columns of symmetry-related stacked molecules. Figure 1 shows that these columns have a stacking sequence of base pair, proflavine, base pair, proflavine, base pair, and so on. The ring nitrogen atoms and the exocyclic amino groups of the proflavine cations face the major groove. The duplex columns are grouped such that diad-

Table 2 Hydrogen bonds involved in pentagon water network in the crystal structure of d(CpG)-proflavine

Pentagons				Branches from pentagons			
Atom i	Atom j	Pentagon	D_{ij} ($\text{\AA}$)	Atom i	Atom j	Pentagon	D_{ij} ($\text{\AA}$)
W3	W20	I	2.91	W3	N7G1	I	2.77
W20	W13	I	3.25	W20	O6G1	I	2.88
W13	W23	I	2.87	W9	W12	I	3.02
W23	W15	I,II	2.41	W13	N10PF1	I,II	2.85
W15	W3	I	2.90	W16	W10	II	2.78
W23	W9	II	2.96	W16	W21	II	2.68
W9	W22	II,III	2.96	W14	N4C1	III,IV	2.91
W22	W16	II	2.75	W6	W24	IV	3.23
W16	W15	II	3.06	W6	N7G2	IV	2.87
W17	W9	III	3.22	W24	O6G2	IV	2.90
W17	W14	III,IV	2.85	W18	N15PF2	IV	2.97
W14	W2	III,V	2.74	W18	O1B	IV	2.66
W2	W22	III	2.76	W25	O1B	IV,V	2.92
W17	W6	IV	2.62	W25	W11	IV,V	2.77
W6	W18	IV	3.15	W11	N10PF2		2.76
W18	W25	IV	3.23	W8	N16PF2	V	2.97
W25	W14	IV,V	2.87				
W25	W8	V	2.78				
W8	O2B	V	2.89				
W2	O2B	V	2.73				

D_{ij} , distance between atoms i and j.

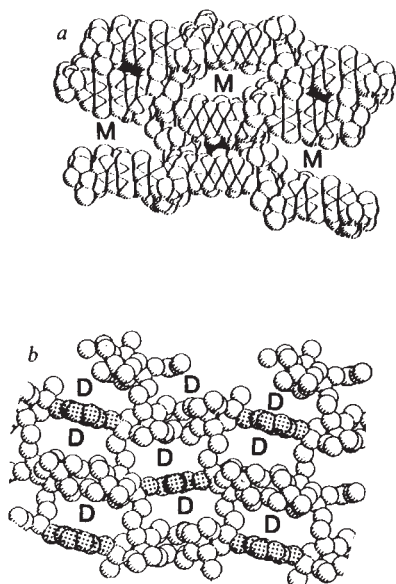


Fig. 1 *a*, Packing of the intercalated duplexes as viewed along the *c* axis. *m*, The major groove cavities; minor groove cavities are shaded. *b*, Water contents of the major and minor groove cavities, also viewed along the *c* axis. The minor groove polygon atoms are shaded; *D* indicates the positions of the intercalated duplexes (shown in *a*).

related pairs, which have their minor grooves facing one another, form minor groove cavities. These alternate with pairs which have their major grooves facing one another forming major groove cavities (Fig. 2). The minor-groove dimers are held together by two hydrogen bonds, and are related to the symmetry-related major groove ones by two (other) hydrogen bonds (see Fig. 2 for details). The minor groove gaps are relatively small and are filled by two-dimensional disk-like assemblies of water molecules, in contrast to the water-filled major groove cavities which are large in two dimensions and extend in infinite channels along the third (Figs 1*b*, 2).

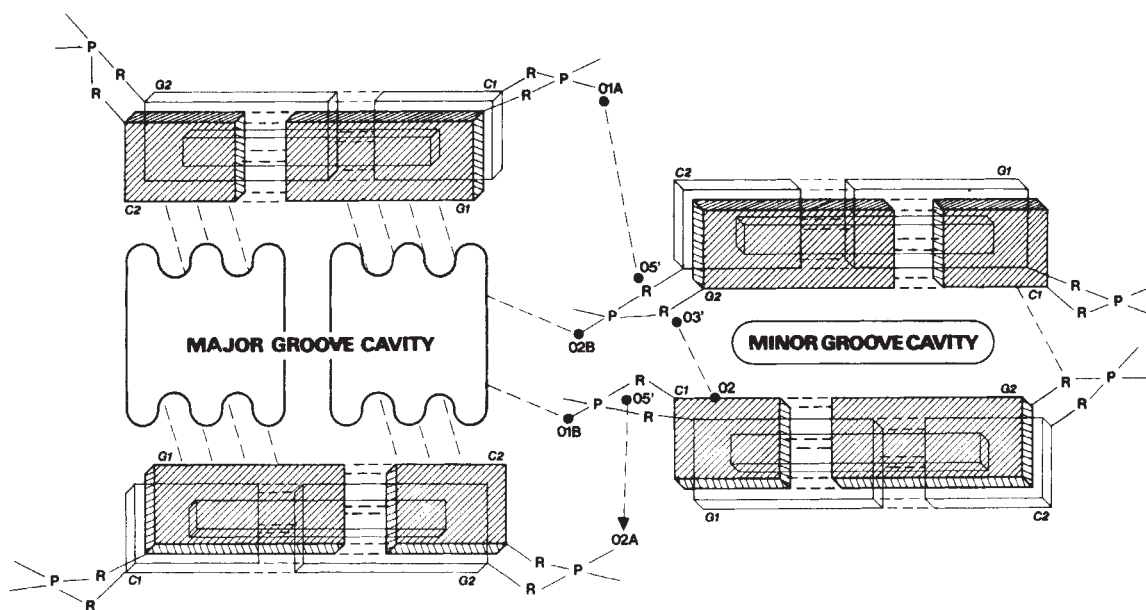


Fig. 2 A schematic illustration of the crystal structure viewed along the *c* axis. *R*, ribose; *P*, phosphate; *C1*, cytidine in strand 1; *C2*, cytidine in strand 2; *G1*, guanine in strand 1; *G2*, guanine in strand 2. Shaded areas indicate the base pairs on top. The dashed lines represent hydrogen bonds. The major and minor groove cavities are shown filled with water.

Structure of the water assemblies

One might suppose that the water molecules in the major groove channels would be disordered, especially as seven of the eight waters per asymmetric unit that do not directly coordinate to the duplexes are located in these channels. However, this is not so. As Fig. 3 shows, the water molecules are linked together in a regular framework, forming edge-linked hydrogen-bonded pentagons. There are four such pentagons per asymmetric unit, each set being joined to the next via water molecule W8. A fifth pentagon is formed by two water molecules (2 and 8) which branch off two pentagons and bridge to a phosphate oxygen of a dinucleoside strand. The major groove channels are thus filled with two diad-related chains of water pentagons, with each chain coordinated to four dinucleoside strands as shown in Fig. 4. The pentagons are constructed to maximize the number of hydrogen-bonding contacts at 3.4-Å intervals along the *c* direction. The water molecules hydrogen bond with the nucleobase atoms in the major groove, as well as with the ring nitrogen atoms and the exocyclic amino groups of the proflavine cations. These hydrogen bonds are all of the 'in-plane variety'¹⁷; that is, the water molecules are in the same plane as the bases and drugs. The arrangement of the pentagons resembles that of the crystal structure of piperazine hexahydrate¹⁸, which has a 'partial pentagonal dodecahedral motif'¹⁹ and is a close relative of the clathrate hydrates¹⁹.

The water arrangements in the small minor groove cavities are precisely one water molecule thick; in each one we see a planar hydrogen-bonded polygon disk of water molecules which in turn hydrogen bond to the atoms in the minor grooves of the bases. Figure 4 shows how, in contrast to the major groove structure, these interactions are 'out of plane'.

Note that in this structure, as in *r*(CpG)-proflavine²⁰, some of the ribose oxygen atoms are hydrated (O1'C1 and O1'G2). Thus, this type of hydration may in fact be the rule rather than the exception as had been believed previously. Another feature of this structure is that not all the possible shell-water coordination has been observed; for example, N4C2 only forms one hydrogen bond and is not hydrated. A similar feature occurs in *r*(CpG)-proflavine but not in the uncomplexed dinucleoside phosphates^{9,10}. It is not yet clear whether incomplete hydration of bases is characteristic of drug-nucleic acid complexes.

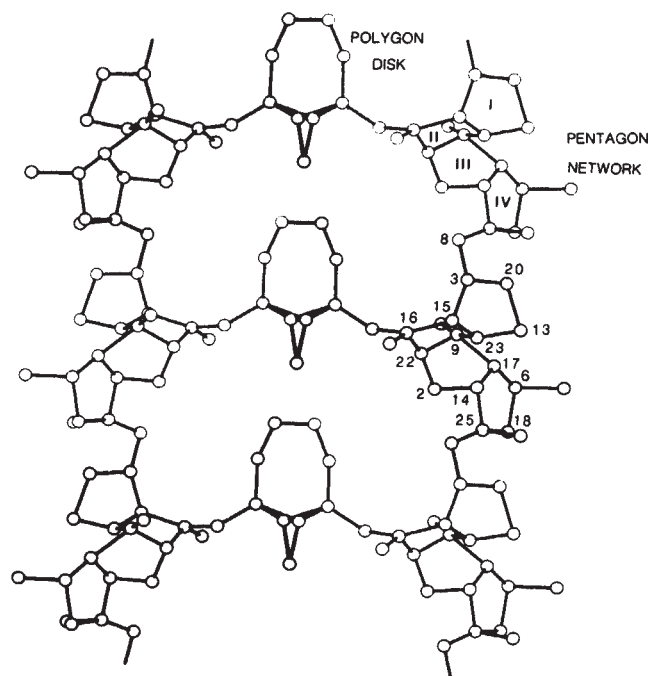


Fig. 3 The water structure alone viewed along the *b* axis. The pentagon network and polygon disk are labelled.

Relationship between water structure and molecular conformation

Comparison of the structures of the more highly hydrated crystal 1 with the less hydrated crystal 2 shows that environmental factors may affect the conformation of a particularly flexible part of a molecule. Crystal 2 is the more highly ordered of the two, both with respect to the conformation of the molecule and the structure of the water network. In this crystal, strand 1 has C3'-endo(3', 5')C3'-endo deoxyribose conformations. Water 7 bridges O3'G1 and a phosphate oxygen, possibly stabilizing the

C3'-endo conformation at the 3' end. Strand 2 has C3'-endo(3'5')C2'-endo deoxyribose conformation (the 'mixed sugar pucker'²¹). In crystal 1, strand 2 has the same conformation as in crystal 2. However, on strand 1 the 3' deoxyribose sugar is disordered, albeit in a specific manner. The significant populations of this disorder are C3'-endo and C2'-endo (each ~50%). In crystal 1, water 7 is not hydrogen-bonded to either of the two disordered O3' hydroxyl positions.

These findings on sugar puckers elucidate further the contentious issue of the role ascribed to sugar conformations in intercalated dinucleosides. Our earlier analysis of a ribo(CpG)-proflavine complex structure²⁰ showed C3'-endo ribose pucker at both ends of the two dinucleoside strands. Even though this structure, by its very existence, showed that intercalation did not necessarily require (or produce) 'mixed sugar' pucker, it has been suggested that proflavine is a special intercalator²¹, presumably on account of the hydrogen bonding of a proflavine cation to the exterior of a duplex via the 3' sugar. Our observation²² that dinucleoside intercalation is concomitant with an increase in the glycosidic torsion angle at the 3' end to a relatively high value suggested that C2'-endo sugar pucker at this end might be slightly favoured in view of the correlation between C2'-endo pucker and high χ values. This deoxy(CpG)-proflavine complex, on the other hand, clearly shows no major preference for either pucker at the 3' end. We conclude that this pucker is unimportant and plays no real part in defining the intercalation geometry. The precise nature of the pucker is dependent on the environment around the sugar and may be considered as an end effect. We would expect the situation in solution to parallel that found in these crystals, with states flipping between the major C2'/C3'-endo puckers to be present.

Discussion

Such highly structured water has never been observed in crystal structures of nucleic acids, their constituents or other biological molecules although circular networks of hydrogen bonds (involving hydroxyl as well as water atoms) have been reported in the structure of α cyclodextrin²³. Although the structures of the nucleic acid fragments, GpC and ApU, are highly hydrated, the waters are evenly distributed in a first hydration shell.

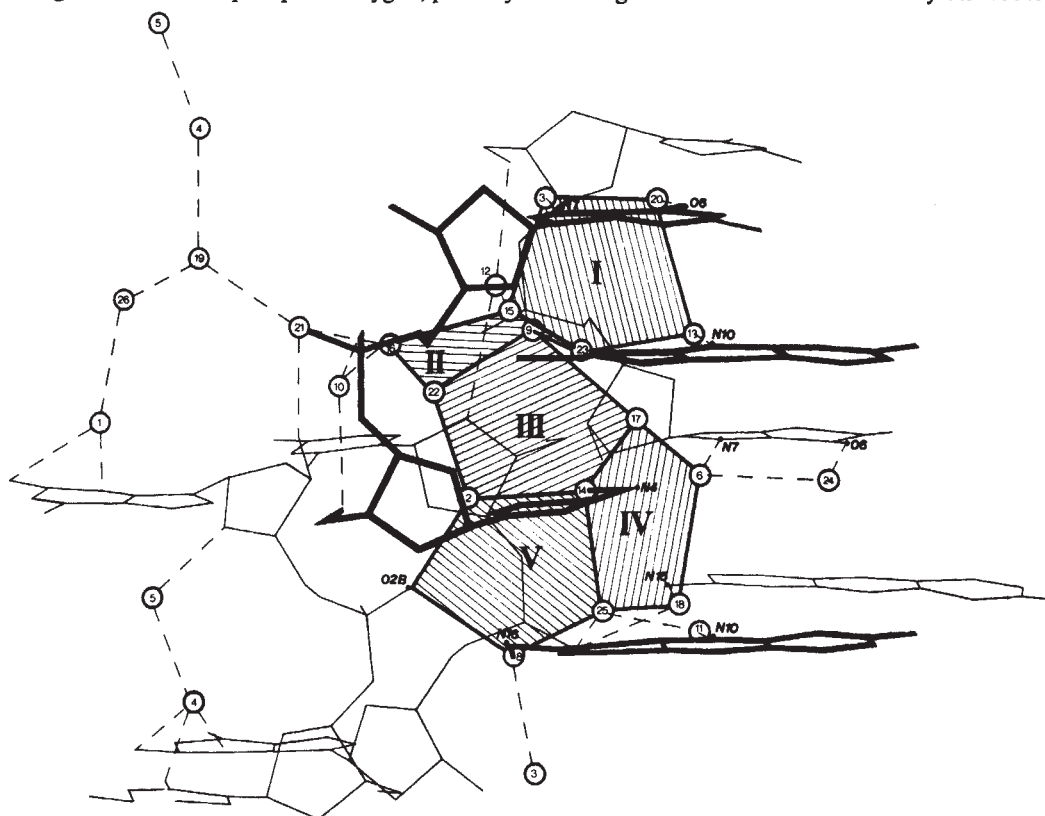


Fig. 4 The hydrogen bonding of the water molecules to the four dinucleoside phosphate strands. The five pentagons are labelled and shaded. The numbering of each water molecule is shown. The view is similar to Fig. 3. Waters 3 and 20 of pentagon I form a square-planar hydrogen-bonding arrangement with N7G and O6G of strand 1, and water 14 hydrogen bonds to N4 of that same strand. Waters 6 and 24, which branch off pentagon IV, form a similar pattern with N7G and O6G of strand 2 of the diad-related duplex. Water 13 of pentagon I hydrogen bonds to N10 of the intercalated proflavine, and water 11, which branches off pentagon IV, hydrogen bonds to N10 of the stacked proflavine. The exocyclic amino groups N15 and N16 of the diad-related stacked proflavine molecules hydrogen bond to waters 18 and 8 respectively.

Two questions arise: (1) Why does this orderly framework appear in this crystal? and (2) is the pentagon network of any wider significance? Although neither question is fully answerable some observations may be made.

The characteristics of the water framework are partially due to the crystal packing. This structure exhibits very few duplex-duplex interactions (Fig. 2), possibly because it contains deoxyribose sugars. It therefore lacks the hydrogen bonding of the O2' hydroxyl which in the most extreme example helped mediate the proflavine-duplex cross-linking observed in proflavine-CpG. Instead, the crystal contains two water-filled cavities. Probably the water disks in the minor groove cavities merely accommodate themselves to the relatively small space allowed by the hydrogen-bonded dimers. The major groove cavities are considerably more hydrophilic with two positively charged nitrogen atoms facing one another. As there are no anions such as SO_4^{2-} mediating these charges, the cavity is necessarily quite large. Solvent-solvent interactions, therefore, become as important as solvent-solute interactions in maintaining the crystal order. In this structure the solvent-solvent interactions take the form of a pentagon network of waters, which can be

likened to a trellis to which the major groove heteroatoms hydrogen bond.

Presumably the pentagon network is not the only plausible arrangement of water molecules—a network of hydrogen-bonded hexagons can also be envisaged. However, aside from the frequent observation of pentagonal water networks in clathrate crystals¹⁹, the geometry observed is such that it clearly forms an optimum number of attachments to drug and dinucleoside atoms. Indeed, the pentagon geometry is well suited to link 3.4-Å-separated heterocycles. Furthermore, the evident propensity of the pentagons to readily associate together and the inherent flexibility of the individual pentagon strongly suggest that nucleic acids of size greater than the dinucleoside level could readily accommodate related pentagonal clusters.

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Amplification of rearranged repeated DNA sequences in cereal plants

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A simple repeating unit of the rye genome, previously known in tandem arrays, has now been shown to occur also in more complex repeating units. One such unit has been cloned and found to contain some sequences unrelated to the simple repeat. It is proposed that tandem arrays of complex repeats are often produced by amplification of DNA segments formed by recombination of different repeats and possibly unique sequences. Some complex variants of repeats are not in high copy number on all rye chromosomes.

REPEATED sequences constitute much of the chromosomal DNA of higher eukaryotes. Many higher plant species have more than 70% of their DNA in the form of repeated sequences¹. Consequently, studies of the formation and organization of families of repeated sequences may contribute substantially to our understanding of plant chromosome structure. Formation of families of repeated sequences must occur often during evolution to account for the differing spectra of repeated DNA sequences in the chromosomes of closely related species². Repeated sequences are frequently reamplified during evolution². This was concluded from comparative studies on repeated sequences common to the genomes of the related graminæ species—wheat, rye, barley and oats². About 20% of the rye genome consists of short, species-specific, repeated sequences interspersed with short repeated sequences also found in wheat and other related cereals^{3,4}. These results suggested that the repeated sequences which are reamplified are often in new combinations which must have been generated by

sequence rearrangement. Studies of the structure of specific repeated sequences of rye heterochromatin have also suggested sequence rearrangement before amplification because each has a simple sub-repeat interspersed with an unrelated non-subrepeating unit⁵. If closely related sequences are amplified in different combinations then different repeating units should have sub-repeats in common.

To investigate this prediction we have probed the wheat and rye genomes with a simple 120-base pair repeating unit that is predominantly organized in tandem arrays⁵ to search for more complex units which are likely to have been created by recombination of the 120-base pair sequence with other sequences before amplification. We describe here the identification, structure and chromosome distribution of such complex forms of this repeat. One of the many complex reamplified forms has been purified by cloning in a bacterial plasmid. Its repeating unit is 2.2 kilobases long and consists of a few of the simple repeat units interspersed with unrelated sequences. The 2.2-kilobase repeat is present on all seven rye chromosomes but other repeats containing sequences related to those in the 2.2-kilobase repeat seem to be present only on some rye chromosomes.

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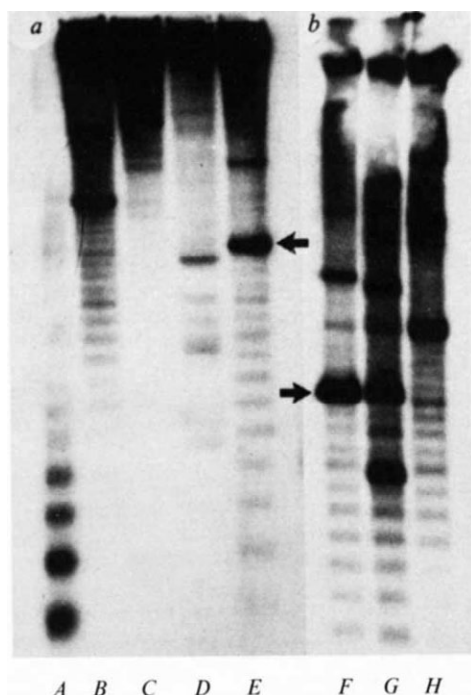


Fig. 1 Hybridization of a cloned repeated sequence to rye DNA digested with various restriction endonucleases. Rye DNA (15 µg) prepared from purified nuclei² was digested to completion *a*, with *Hae*III (A), *Bam*HI (B), *Bgl*II (C), *Hind*III (D), *Eco*RI (E) and *b*, with *Eco*RI (F), *Eco*RI + *Bam*HI (G) and *Bam*HI (H), and fractionated by agarose gel electrophoresis. The agarose concentration in the gel was 1.5%. Electrophoresis conditions were as described previously⁵. Electrophoresis was from top to bottom of the panel. The DNA was transferred from the gel to a sheet of nitrocellulose filter as described by Southern¹⁹. The filter was hybridized using a ³²P-labelled cRNA probe prepared *in vitro* by *E. coli* RNA polymerase using a cloned example of a 120-base pair highly repeated DNA sequence as the template (see text for details of repeat). 5×10^5 c.p.m. were used in the hybridization. Hybridization was in $2 \times$ SSC containing 100 µg tRNA at 62 °C for 18 h. (SSC is 0.15 M sodium chloride + 0.015 M sodium citrate). Unhybridized probe was removed from the filters by extensive washing with $1 \times$ SSC at 60 °C. Stable hybridization was revealed by autoradiography. The major 2.2-kilobase repeats in the *Eco*RI digests are arrowed.

Complex forms of a simple tandem repeat

The family of rye repeats we have analysed for more complex reamplified forms comprises ~2% of the genome⁵. It consists principally of 120-base pair repeating units arranged in tandem arrays. This was determined by digesting rye DNA with *Hae*III restriction endonuclease, fractionating the resulting fragments by agarose gel electrophoresis, transferring the fragments to a nitrocellulose filter and hybridizing them with ³²P-labelled cRNA prepared from a cloned 120-base pair repeat. (The cloning of this repeat unit in the bacterial plasmid pBR322 is described elsewhere⁵.) *Hae*III digestion (Fig. 1, track A) revealed a progression of fragments at intervals of 120 base pairs. This pattern would be expected from a family of tandemly arranged repeats in which each unit repeat sequence contains a *Hae*III site but occasionally loses it by random mutation so that some dimers, trimers and higher multimers remain after *Hae*III digestion. Figure 1 also shows that the restriction enzymes *Bam*HI (track B), *Bgl*II (track C), *Hind*III (track D) and *Eco*RI (track E) cut sequences complementary to the 120-base pair repeat probe DNA infrequently. *Bam*HI and *Eco*RI produce an integral series of fragments with a periodicity of 120 base pairs. Such fragments presumably arise from the creation of restriction sites by mutation. *Bgl*II and *Hind*III do not generate such an integral series of fragment lengths. Therefore there is probably no sequence closely related to the *Bgl*II and *Hind*III recognition sites within the basic repeating unit. That *Bam*HI and *Eco*RI sites occur randomly in the 120-base pair family is supported by double-enzyme digestion. Digestion with both *Eco*RI and *Bam*HI (Fig. 1, track G) produces a higher proportion of the family in lower molecular weight fragments than does

digestion with either *Eco*RI (Fig. 1, track F) or *Bam*HI (Fig. 1, track H) alone.

The results in Fig. 1 (tracks B–E) also show that the clone of the 120-base pair unit repeat hybridizes to specific restriction fragments which are in relatively much higher copy number than the integral progression of 120-base pair fragments. All hexanucleotide restriction enzymes used produce such fragments. These fragments could have arisen in the genome by (1) amplification of specific arrays of 120-base pair units, one member containing a particular hexanucleotide recognition site, or (2) the amplification of more complex pieces of DNA that include 120-base pair units. That at least some of these major variant forms contain sequences other than 120-base pair units is suggested by their being present in *Hind*III and *Bgl*II digests, even though these enzymes do not produce an integral series of 120-base pair fragments. We have shown that the major variant revealed in the *Eco*RI digests (arrowed in Fig. 1) does contain a complex piece of DNA besides 120-base pair units. This was achieved by characterizing cloned examples of the major variant.

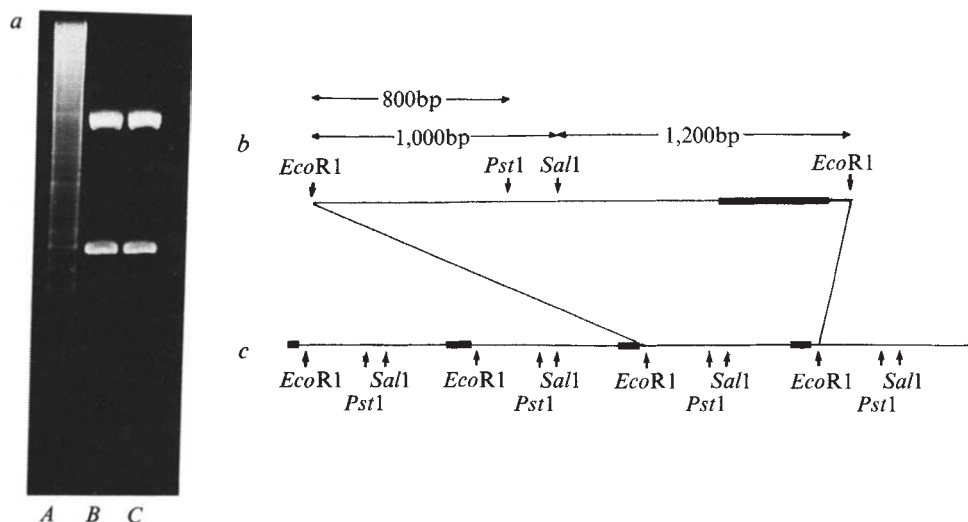
Major *Eco*RI variant is not a simple tandem array of 120-base pair units

The major high copy number variant in *Eco*RI digests is 2.2 kilobases long and migrated with a DNA band of strong fluorescence in *Eco*RI digests of total rye DNA (Fig. 2a). This band was recovered from an agarose gel and cloned in the vector plasmid pACYC184. The resulting clones were screened with the 120-base pair sequence by the method of Grunstein and Hogness⁶. Two clones that hybridized with the 120-base pair repeat sequence and co-migrated with the major band in *Eco*RI digests of *Secale cereale* DNA were obtained (Fig. 2a). A physical map of one of the cloned 2.2-kilobase rye DNA fragments is shown in Fig. 2b. The sequence contains sites for endonucleases *Pst*I and *Sal*I. The 120-base pair repeat unit hybridizes only to the 1,200-base pair *Sal*I-*Eco*RI fragment and the 1,400 base pair *Pst*I-*Eco*RI fragment and not to the 1,000-base pair *Sal*I-*Eco*RI fragment or the 800-base pair *Pst*I-*Eco*RI fragment (results not shown). The 120-base pair repeat unit hybridizes to only three *Hae*III fragments of 220, 200 and 120 base pairs which map within the 1,200-base pair *Sal*I-*Eco*RI fragment. Thus it was deduced that the 120-base pair repeat unit in the high copy *Eco*RI variant is interspersed with unrelated sequences, all the sequences together forming a unit repeat defined by *Eco*RI, *Pst*I or *Sal*I. For ease of discussion we shall refer to the DNA unrelated to the 120-base pair unit as 'spacer' DNA. The 2.2-kilobase repeat units are clustered in rye chromosomes because dimer and trimer forms can be seen in partial *Eco*RI digests of rye DNA. Fig. 2c illustrates the deduced arrangement of the 2.2-kilobase repeat in the genome.

The 2.2-kilobase repeat unit family may have been formed later than the 120-base pair repeat unit family

Two lines of evidence suggest that the 2.2-kilobase repeat unit family may have been formed in the rye genome more recently than the 120-base pair repeat unit family. First, in complete digests of rye DNA >80% of the 2.2-kilobase repeat DNA is in the monomer form after digestion with *Eco*RI, whereas <40% of the 120-base pair repeat DNA is in the monomer form after complete digestion with *Hae*III (Fig. 1). Assuming that dimer, trimer and such forms result from the random loss of restriction sites with time, and that each base has an equal probability of being altered, then more of the six-base pair *Eco*RI sites would be expected to have been lost than four-base pair *Hae*III sites if the families were of equal age. Second, the 2.2-kilobase repeat family is not highly repeated in wheat (see below; Fig. 5) whereas the 120-base pair family is⁵. This suggests that the 120-base pair family was amplified before wheat and rye diverged from their common ancestor², and the 2.2-kilobase repeat family was amplified after the rye and wheat ancestors diverged,

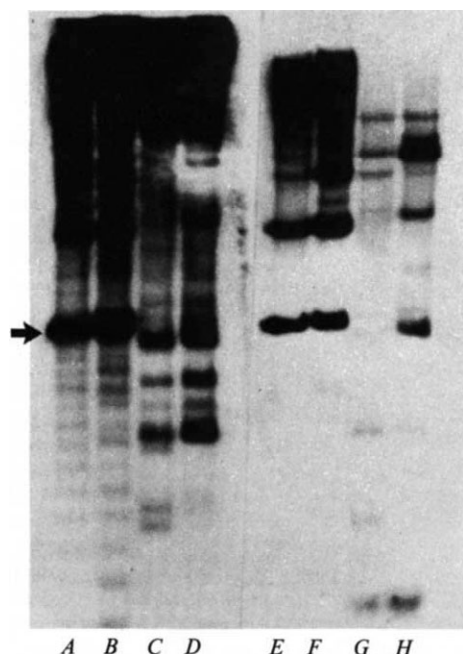
Fig. 2 The structure of a cloned example of a complex form of the 120-base pair repeat. Rye DNA (100 µg) was digested to completion with *EcoRI* and fractionated on a 1.5% low melting point agarose (LMP Seakem) gel. The major repeat band in this digest (see *a*, lane A) was excised and heated at 65 °C together with 2 vol (w/v) 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA for 3 min. The solution was cooled to 37 °C and an equal volume of water-saturated phenol (at 37 °C) was added. The mixture was homogenized, allowed to equilibrate to room temperature and the phases separated by centrifugation at room temperature. The phenol phase was removed and the aqueous phase re-extracted with phenol. The final aqueous phase was precipitated with 2.5 vol ethanol. The precipitated DNA was washed twice with 70% ethanol, dried and dissolved in 20 µl H₂O. The DNA was ligated to 0.1 µg *EcoRI*-linearized pAC184 DNA⁵. The ligated DNA was used to transform the *E. coli* strain HB101. Transformants were selected on media containing tetracycline at 5 µg ml⁻¹. Transforming plasmids containing inserted fragments were screened by testing for chloramphenicol sensitivity. These transformants were then screened for sequences complementary to the 120-base pair repeat using the assay described by Grunstein and Hogness⁶. Plasmid DNA from such transformants was prepared²⁰, digested with *EcoRI* and fractionated by agarose gel electrophoresis together with rye DNA. *a*, Track A shows rye DNA, tracks B and C show two plasmids. *b*, A physical map of one such plasmid. The double thickness line shows the region complementary to the 120-base pair repeat. This was determined by hybridizing cRNA prepared from a cloned example of the 120-base pair repeat to the plasmid illustrated in 2*a* (track B), digested with *EcoRI* + *SalI*, *EcoRI* + *PstI*, and *EcoRI* + *HaeIII*, fractionated by gel electrophoresis and transferred to nitrocellulose paper. We have assumed that the three *HaeIII* fragments (see text) which hybridize to the clone of the 120-base pair repeat are adjacent. The region has been positioned as illustrated because the 200-base pair fragment carries the *EcoRI* site. *c*, The deduced structure of the complex cloned repeat (the 2.2-kilobase repeat, see text) in the genome. The double thickness line represents that portion of the repeat assumed to include sequences complementary to the 120-base pair repeat. bp, Base pairs.



although we cannot rule out the possibility that the 2.2-kilobase repeat has been deleted from wheat.

Origin of the 2.2-kilobase repeat family

To investigate the arrangement of the spacer DNA sequences in *Secale* genomes, a probe prepared from DNA fragments within the 2.2-kilobase spacer DNA (the 800-base pair *EcoRI*-*PstI* fragment) was hybridized to size-fractionated *Secale cereale* (rye) and *Secale silvestre* DNAs digested with *EcoRI* and *HindIII* endonucleases. Figure 3 (tracks E-H) shows that the spacer DNA hybridizes to many repeated sequence DNA bands in DNAs digested with *EcoRI* or *HindIII*. The pattern of hybridization produced when the 120-base pair repeat DNA was used as probe is shown as a control (Fig. 3 A-D). The spacer DNA probe hybridized to DNA fragments containing from >20



kilobases to >0.5 kilobases. This hybridization pattern suggests that sequences related to those in the 2.2-kilobase spacer DNA exist in many diverse arrangements in the genome. This diversity and the origin of the 2.2-kilobase repeat can be explained by proposing that short pieces of DNA frequently undergo rearrangement by recombination during genome evolution to form many permutations of linear order. Specific permutations of these sequences are then amplified to create repeat families, such as the 2.2-kilobase repeat family, definable by their unique length and linear permutation of rearranged sequences.

The 2.2-kilobase repeat contains two components that we have distinguished—the 120-base pair sequences and what we have called 'spacer sequences'. Two pieces of evidence suggest that these subcomponents have existed as repeats in cereal genomes for long evolutionary time periods. First, much of the 2.2-kilobase spacer DNA is present in rye, wheat and barley genomes because most (but not all) of the 800-base pair *EcoRI*-*PstI* fragment of the 2.2-kilobase repeat (see Fig. 2*b*) renatures with repeated sequence kinetics when sheared and renatured with a large excess of sheared wheat and barley DNAs⁷. This suggests that these sequences were first amplified before wheat, rye and barley diverged². The presence of the 120-base pair sequence in wheat is shown in Fig. 5 and has also been demonstrated by *in situ* hybridization to wheat chromosomes⁵. Second, sequences in the rye genome related to the subcomponents of the 2.2-kilobase repeat are very heterogeneous. This was determined by forming hybrid duplexes *in vitro* between rye DNA fragments and a cloned example of 120-base pair repeats or the 800-base pair *EcoRI*-*PstI* fragment from the 2.2-kilobase repeat. The thermal stabilities of these duplexes were

Fig. 3 Organization of spacer DNA from the 2.2-kilobase repeat in *Secale* species DNA. The 800-base pair *EcoRI*-*PstI* fragment (see Fig. 2*b*) was prepared from preparative *EcoRI* + *PstI* double digests of the cloned 2.2-kilobase repeat fractionated on agarose gels as described in Fig. 2 legend. ³²P-labelled cRNA prepared from this fragment was hybridized to *S. cereale* (rye) DNA digested with *EcoRI* (E) or *HindIII* (G) and *S. silvestre* DNA digested with *EcoRI* (F) and *HindIII* (H). As a control, ³²P-labelled cRNA prepared from the 120-base pair repeat was hybridized to *S. cereale* digested with *EcoRI* (A) or *HindIII* (C) and to *S. silvestre* DNA digested with *EcoRI* (B) or *HindIII* (D). The DNA was fractionated by electrophoresis on 1.0% agarose gels (from top to bottom) and transferred to nitrocellulose filters before hybridization. Hybridization and washing conditions were as in Fig. 1 legend. Hybridization was assayed by autoradiography. The major 2.2-kilobase repeats are arrowed.

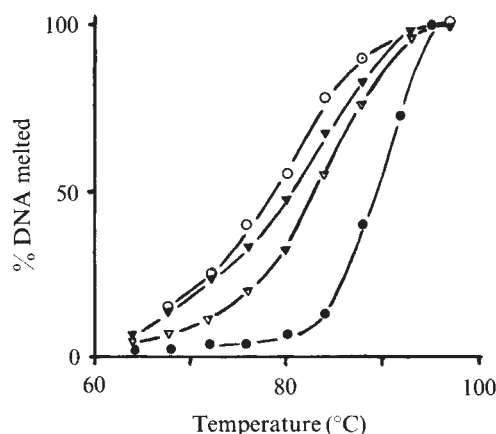


Fig. 4 Thermal stability of duplexes formed *in vitro* between rye DNA and the 120-base pair or 2.2-kilobase spacer DNA. DNA from a clone of the 120-base pair repeat or the 800-base pair *EcoRI*-*PstI* fragment from 2.2-kilobase spacer, radiolabelled with ^{32}P by nick translation (10^5 c.p.m. of specific activity, 10^8 c.p.m. μg^{-1}) and was mixed with 200 μg rye DNA sheared to 300–400 nucleotide lengths. After denaturation the DNA was incubated to C_0t 0.3 in 0.12 M phosphate buffer at 60°C. The renatured DNA was adsorbed on to hydroxyapatite at 60°C in 0.12 M phosphate buffer, then melted off by raising the temperature in defined steps. Denaturation of total rye repeated DNA was determined by measuring the elution of A_{260} . Denaturation of the labelled DNA duplexes was determined by precipitating the eluted DNA with 5% TCA in the cold, collecting it on GF/B filters and scintillation counting. Sheared native rye DNA was similarly eluted as a control. $\circ$, 2.2-kilobase spacer duplexes; $\blacktriangledown$, 120-base pair DNA duplexes; ∇ , total renatured repeated rye DNA; $\bullet$, native rye DNA. Results plotted are means from two experiments.

determined by binding them to hydroxyapatite and melting them off by raising the temperature in defined steps. Both sets of duplexes show a very broad melting curve with a ΔT_m of $\sim 11^\circ\text{C}$ with respect to native DNA (Fig. 4). A broad melting curve is expected of hybrids with a large range of mispaired bases. These melting curves therefore indicate considerable heterogeneity amongst sequences related to the 120-base pair and spacer DNA. Assuming that repeated sequence divergence is related to the age of the repeated sequence family, sequences related to the 120-base pair and to spacer DNA have existed as repeats for a long and similar time period. The sequence heterogeneity of

these families contrasts with the relative homogeneity of a family described elsewhere which is specific to *S. cereale* and absent from *S. silvestre* and which was amplified presumably relatively recently⁵.

The hybridization experiments of Fig. 3 provide evidence that both subcomponents of the 2.2-kilobase repeat have been involved in rearrangements. Probes prepared from both the 120-base pair sequence and the spacer sequence hybridize to a smear of DNA fragments ranging in size from >20 to 0.5 kilobases suggesting that sequences complementary to these DNAs are interfaced with many different sequences in the chromosomes. Figure 3 also shows that many permutations of the ancestral sequences are reamplified. The two subcomponents of the 2.2-kilobase repeat hybridize to many bands other than the integral size progression of the 120-base pair tandem repeat. Furthermore, several of the bands (other than the 2.2-kilobase repeat itself) which hybridize to the two subcomponents of the 2.2-kilobase repeat have the same length. This suggests that sequences related to the 120-base pair and spacer DNA sequences have been amplified together to form repeat families differing from the 2.2-kilobase repeat family. DNA sequences homologous with 2.2-kilobase spacer DNA have been involved in amplification (and possibly rearrangement) events since the separation of *S. cereale* and *S. silvestre* because repeated DNA bands visible in hybridizations to *S. cereale* (Fig. 3, tracks E and G) are absent from the hybridizations to *S. silvestre* (Fig. 3, tracks F and H) and vice versa.

The heterogeneity of the sequences in the genome related to the subcomponents of the 2.2-kilobase family makes it unlikely that these sequences have been rearranged or translocated to new sites in the genome as 'intact, mobile' elements such as a bacterial insertion sequence⁸ or the copia, 412 or 279 elements in *Drosophila*⁹. If spacer DNA always inserted itself intact, all the *HindIII* restriction fragments from the genome which hybridize to spacer DNA would be expected to be larger than the spacer itself because spacer DNA in the 2.2-kilobase repeat does not contain a *HindIII* recognition sequence. The results in Fig. 3 (tracks G and H) show three repeat families in *S. cereale* and two in *S. silvestre* which hybridize to 2.2-kilobase spacer DNA but which are shorter than the calculated size of the 2.2-kilobase repeat spacer. Thus spacer DNA must at least undergo deletion or be the target of insertion itself by a DNA sequence containing a *HindIII* recognition sequence.

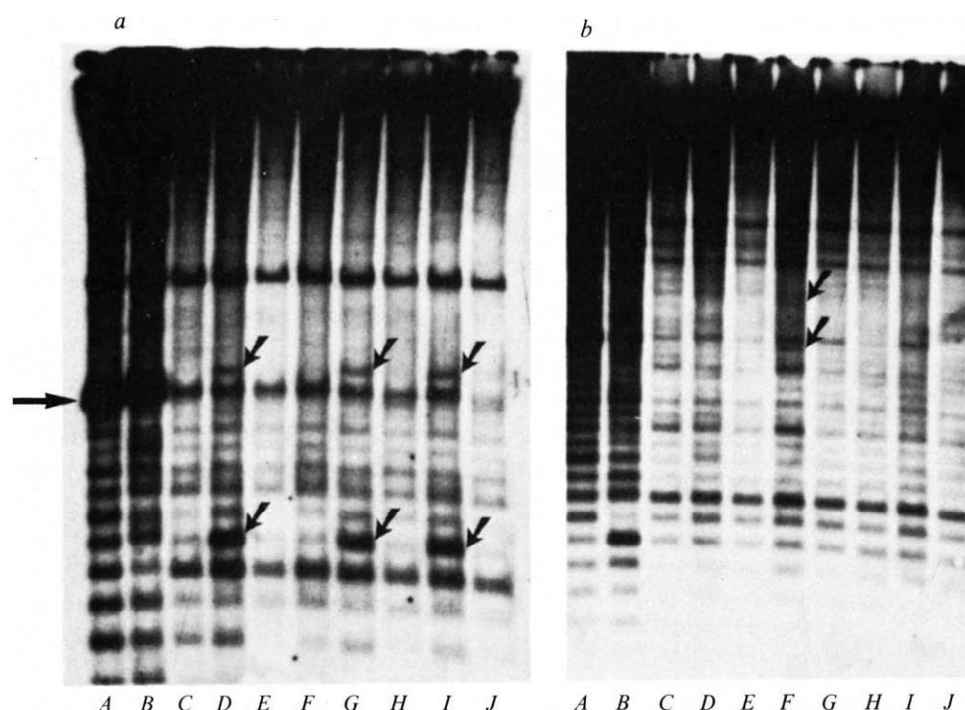


Fig. 5 The presence of complex forms of the 120-base pair repeat in individual chromosomes of rye. DNAs from rye (variety King II), *S. silvestre* and *Triticum aestivum* (variety Holdfast) and the complete set of alien addition lines of the *S. cereale* variety King II in the *T. aestivum* variety Holdfast were digested with *EcoRI* (a) and *BamHI* (b) and fractionated by electrophoresis on 1.5% agarose gels. The fractionated DNA was transferred to nitrocellulose filters and hybridized with ^{32}P -labelled cRNA prepared using a cloned example of the 120-base pair repeat. Hybridization was assayed by autoradiography. Track A, rye DNA; track B, *S. silvestre* DNA; track C, *S. cereale* chromosome 5R in a *T. aestivum* background DNA; track D, chromosome 6R; track E, chromosome 2R; track F, chromosome 4R; track G, chromosome 1R; track H, chromosome 3R; track I, chromosome 7R; track J, *T. aestivum* DNA. The major 2.2-kilobase repeats revealed by *EcoRI* digestion are indicated by the arrow at the extreme left. The other arrows mark bands which vary in different addition line tracks.

Chromosomal distribution of reamplified variants of the 120-base pair repeat

Wheat-rye addition lines have enabled us to investigate quantitative variation of specific reamplified variants of the 120-base pair repeat between the chromosomes of the rye variety King II. Each wheat-rye addition line has a full complement of wheat chromosomes and a single pair of rye chromosomes¹⁰. DNA isolated from each of the addition lines, from King II rye, from *S. silvestre* and from wheat (variety Holdfast) were digested with *Eco*RI (Fig. 5a) or *Bam*HI (Fig. 5b) restriction endonucleases, fractionated by gel electrophoresis, transferred to nitrocellulose paper and hybridized with a probe prepared from the 120-base pair repeat. The results in Fig. 5 show that *S. cereale* (rye) (track A) and *S. silvestre* (track B) have essentially the same set of amplified variants of the 120-base pair repeat. Wheat DNA (track J), however, lacks a high concentration of the major variant in the *Eco*RI digest. It is therefore possible to use the addition lines to investigate the chromosomal distribution of the rye 2.2-kilobase repeats. Figure 5a shows that the 2.2-kilobase family is present in each of the seven rye chromosomes examined as is the major family in *Bam*HI digests (Fig. 5b). However, two variants in *Eco*RI digests are present in high copy number only in chromosomes 1R, 6R and 7R (Fig. 5a, arrowed in tracks D, G and I) and one or two relatively minor variants in *Bam*HI digests (Fig. 5b, arrowed in track F) may be present in high copy number only in chromosome 4R.

That some amplified variant forms of the 120-base pair repeat may be chromosome specific or on only some chromosomes suggests that specific amplified sequences may occur initially at a specific location in the genome and are only subsequently distributed to other chromosomes by recombination.

Discussion

High copy number variants within families of repeated sequences have been found in a few other species by methods similar to ours—in satellite DNAs of mouse¹¹, *Drosophila*¹² and man^{13–15}. Only for *Drosophila*^{12,16,17} and man^{13–15}, as far as we

are aware, have results been previously reported which illustrate chromosome-specific variants of repeated sequences. An intriguing question is whether chromosome-specific variation is maintained for functional reasons or merely reflects the site of reamplification before dispersal¹⁴.

The hypothesis described here to account for the origin of the complex 2.2-kilobase family of repeated sequences is relevant to the structure and evolution of a large proportion of plant chromosome DNA because complex permutations of short repeated and single-copy sequences are common in plant chromosomes¹⁸. Furthermore, in cereal species, a large fraction of the DNA consists of short repeated sequences common to many species, interspersed with repeated sequences which are species-specific or found only in very closely related species^{3,4}. DNA with this sequence arrangement has probably evolved by sequence rearrangement and amplification like the 2.2-kilobase repeat family described here.

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Cloning and sequence analysis of cDNAs encoding two distinct somatostatin precursors found in the endocrine pancreas of anglerfish

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Complementary DNAs for two distinct anglerfish somatostatin peptides (termed I and II) have been cloned in bacterial plasmids and sequenced. The nucleotide sequence for somatostatin I encodes a large precursor peptide (molecular weight 13,300) in which the somatostatin hormone is at the carboxyl terminus. The predicted 14-amino acid sequence for anglerfish somatostatin I is the same as mammalian somatostatin. Somatostatin II is also synthesized as part of a larger precursor (molecular weight 14,100) with the presumptive somatostatin hormone also at the carboxyl terminus. The 14-amino acid sequence of somatostatin II differs from somatostatin I at two internal residues (Tyr in place of Phe 7 and Gly in place of Thr 10). The two different somatostatins may have distinct biological activities. Homologies in the amino acid sequences of the two peptides outside the somatostatin moiety suggest other regions of the molecules have biological functions.

SOMATOSTATIN is a 14-amino acid peptide which modulates the secretion of several peptide hormones and may also have a role in neurotransmission. Somatostatin was originally isolated from the hypothalamus as an inhibitor of pituitary growth hormone secretion¹, but it has more recently been localized in

the D cells of the endocrine pancreas^{2,3} and shown to be an inhibitor of both insulin and glucagon secretion⁴. In addition, it has been detected in discrete epithelial cells lining the gut⁵ and can inhibit the secretion of several gastrointestinal hormones including secretin⁶, gastrin⁷ and cholecystokinin⁸. However,

somatostatin is not universally involved in regulating peptide hormone secretions because it does not affect the secretion of several other hormones including follicle-stimulating hormone and luteinizing hormone⁹. Studies on the localization of somatostatin in neuronal tissue reveal its presence in several other regions of the brain^{10,11} as well as the spinal cord¹² and the sympathetic nerve fibres¹³. Injection of somatostatin into the cranium results in loss of motor activity, suggesting that it is a potent depressant of certain parts of the central nervous system¹⁴. These properties have led to the postulate that somatostatin is a neurotransmitter. That a single hormone exhibits this spectrum of biological activities is a regulatory paradox.

These observations have engendered considerable interest in the regulation of somatostatin synthesis and secretion by the various dispersed somatostatin-producing cells. In common with several other peptide hormones, somatostatin is synthesized as part of a larger precursor peptide that is subsequently processed^{15,16}. Peptides up to 10 times the size of somatostatin have been found which react with somatostatin antibodies^{17,18} and there is evidence that this larger peptide is also able to inhibit growth hormone secretion by the pituitary¹⁹. Furthermore, a 28-residue peptide containing somatostatin has recently been isolated from porcine intestinal tissue²⁰.

A direct approach to characterizing the complete somatostatin precursor, as well as the somatostatin mRNA and gene, involves construction of bacterial plasmids containing the somatostatin mRNA sequence, using nucleic acid technology. This approach is facilitated by the availability of a tissue which is highly enriched in somatostatin mRNA. In most vertebrates, there is no tissue with a sufficiently high proportion of somatostatin-producing cells to undertake such experiments. However, in some fish, and in particular the anglerfish (*Lophius americanus*), the endocrine pancreas (termed Brockmann body) is easily separated from exocrine tissue and, furthermore, in comparison with mammals, is highly enriched in D (somatostatin-producing) relative to B (insulin-producing) cell types²¹. Therefore, we have used the anglerfish Brockmann body as a source of somatostatin mRNA.

We present here the results of cloning in bacteria and subsequent sequence analysis of a cDNA molecule which predicts the complete preprosomatostatin peptide sequence (somatostatin I) synthesized by the fish endocrine pancreas. In addition, we present the sequence of another cDNA molecule, also present in the fish endocrine pancreas, encoding a second preprosomatostatin precursor (somatostatin II) which differs by two amino acids in the 14-residue hormone sequence. The finding that two distinct precursor peptides are synthesized in the fish pancreas leads us to suggest that more than one gene may be involved in the hormonal activities now attributed to somatostatin.

Synthesis and cloning of fish cDNA

Poly(A)⁺ mRNA from Brockmann body tissue was isolated as described in Fig. 1 legend. This fraction contains two major classes of mRNA: 700 ± 25 and 840 ± 25 bases. This is consistent with a previous report that *in vitro* translation of the mRNA from anglerfish Brockmann bodies results in only a few predominant protein products²². Double-stranded cDNA was synthesized from poly(A)⁺ mRNA, tailed with deoxycytidine residues and cloned in the *Pst*I site of the plasmid pBR322 which had been previously tailed with deoxyguanosine (described in Fig. 1 legend). Approximately 3,000 tetracycline-resistant transformants were obtained from 75 ng of tailed cDNA.

To screen the transformants, the most frequent cDNA inserts were selected by hybridization to a single-stranded cDNA probe synthesized from Brockmann body poly(A)⁺ RNA. A few plasmids containing large inserts were prepared from the cDNA-positive colonies. Purified inserts from these plasmids were labelled by 'nick translation' and tested for their ability to cross-hybridize with each other. The most frequent cDNA insert among the colonies selected by this method has been subsequently sequenced and found to be fish preproinsulin²³.

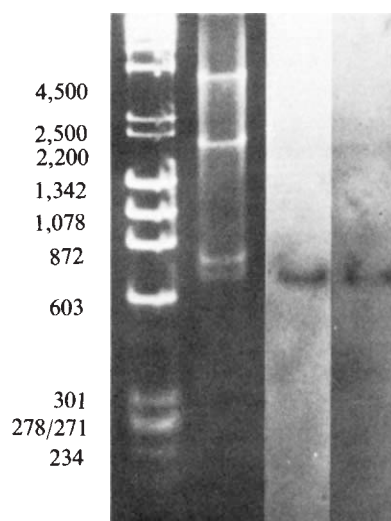
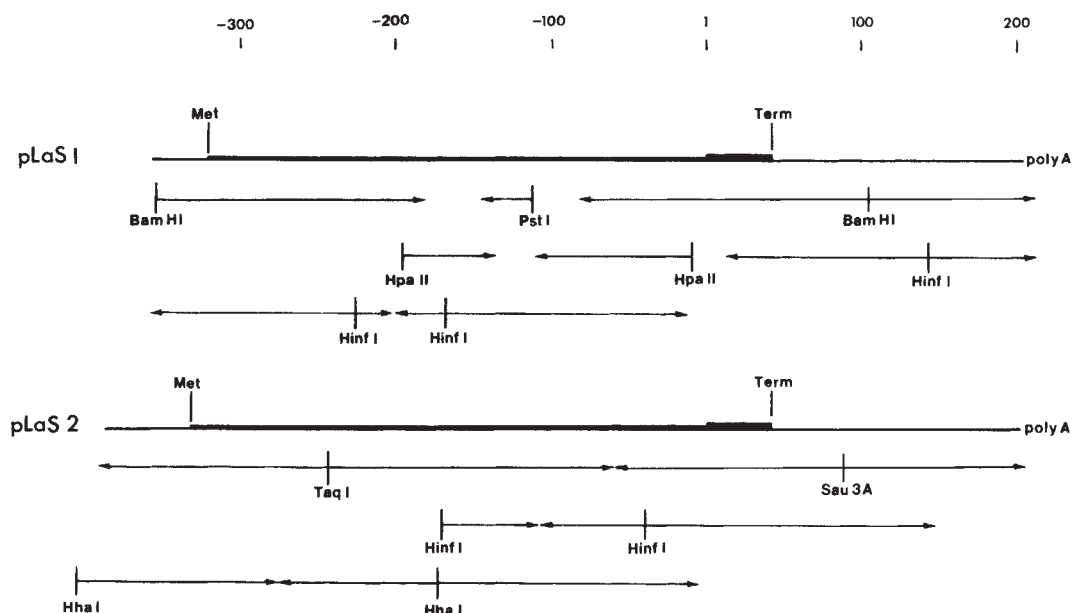


Fig. 1 RNA was extracted from anglerfish Brockmann bodies (Biological Associates) by the guanidine thiocyanate method²³. One gramme of Brockmann body tissue yields approximately 6.5 mg of total RNA. Polyadenylated RNA was purified using oligo(dT)-cellulose affinity chromatography. 1.35 μ g of single-stranded cDNA was made from 10 μ g polyadenylated RNA using AMV reverse transcriptase (J. Beard, lot no. 1577) in the following reaction mix; 40 mM Tris-HCl, pH 8.3, 10 mM MgCl₂, 56 mM β -mercaptoethanol, 0.5 mM dATP, dGTP, dTTP, 0.15 mM ³H-dCTP (specific activity 5.2 mCi μ mol⁻¹), 25 μ g ml⁻¹ oligo(dT) (P-L Biochemicals), 40 μ g ml⁻¹ poly(A)⁺ RNA, 15 units reverse transcriptase per μ g RNA. Incubation was at 42 °C for 30 min and then at 68 °C for 2 min. The reaction was stopped by the addition of NaEDTA (pH 8.0) to 20 mM, chromatographed over Sephadex G-75 and precipitated with ethanol. RNA was removed by alkali treatment (0.2 M NaOH, 23 min at 68 °C) and the second strand cDNA synthesis was carried out in the following reaction mixture using DNA polymerase I; 100 mM Na HEPES pH 6.9, 75 mM KCl, 10 mM MgCl₂, 10 mM dithiothreitol (DTT), 100 mM dNTPs, 0.8 μ g ml⁻¹ single-stranded cDNA (boiled for 3 min just before addition), and 75 units per μ g *Escherichia coli* DNA polymerase I (Biolabs). Incubation was at 14 °C for 2 h. The reaction volume was made 20 mM NaEDTA, extracted once with phenol/chloroform/isoamyl alcohol (50:48:2) and passed over a Sephadex G-75 column. In preparation for cDNA tailing, the hairpin loops of the double-stranded DNA (ds-cDNA) were clipped and made blunt ended using S₁ micrococcal nuclease (Miles) in a 30-min reaction at 37 °C (175 units per μ g ds-cDNA). After organic extraction and Sephadex G-75 chromatography to remove small DNA fragments, 300 ng of trimmed double-stranded (ds)-cDNA was tailed using calf thymus terminal transferase in a 30-min reaction at 37 °C containing 140 mM K cacodylate, 30 mM Tris base (pH 7.8), 1 mM CoCl₂, 0.1 mM DTT, 25 μ M dCTP (α -³²P)dCTP, 8.36 mCi nmol⁻¹, and 150 units terminal transferase per μ g ds-cDNA³⁴. Preliminary experiments indicated approximately 30 bases are added to the 3' end in these conditions. The dC-tailed ds-cDNA was then annealed to an equimolar amount of dG-tailed pBR322 at a concentration of 1 μ g ml⁻¹ using sequential 2 h incubations at 42, 30 and 14 °C (ref. 35). The hybrid plasmid DNA was precipitated with ethanol and used to transform *E. coli* λ 1776 in P2/HV2 containment conditions in compliance with NIH Recombinant DNA Guidelines³⁶ and selected for tetracycline resistance. To screen with various labelled probes, recombinant colonies were grown on Whatman 541 cellulose paper, lysed *in situ* and prepared for hybridization according to the Grunstein and Hogness procedure³⁷. The filters were hybridized to a ³²P-labelled cDNA probe synthesized from anglerfish Brockmann body RNA and plasmid DNA was isolated from those colonies giving a strong autoradiographic signal using a cleared lysate procedure³⁸. Two plasmids (pLaS1 and pLaS2) were selected on the basis of their size and their cDNA inserts purified on preparative acrylamide gel electrophoresis. Purified insert cDNA from these plasmids was labelled by 'nick translation'³⁹ and hybridized to anglerfish poly(A) RNA which had been previously chromatographed on a 2% agarose methyl mercury gel⁴⁰ and transferred to diazotized cellulose paper²⁴. Hybridization was for 48 h at 42 °C. Hybridized filters were autoradiographed after washing three times (100 ml volume, 10 min each) in 0.3 M NaCl, 0.03 M Na citrate, 0.1% (w/v) SDS at 20 °C and then in the same manner using 15 mM NaCl, 1.5 mM Na citrate, 0.1% SDS at 50 °C. Lane 1, *Hind*III-digested λ DNA and *Hae*III-digested Φ X174 DNA MW standards. Lane 2, ethidium bromide staining of anglerfish Brockmann body (1 μ g poly(A)⁺-enriched RNA); lane 3, hybridization of transferred anglerfish poly(A)⁺ RNA to pLaS1 ³²P insert DNA; lane 4, hybridization of transferred anglerfish poly(A)⁺ RNA to pLaS2 ³²P insert

Fig. 2 Sequencing strategy for pLaS1 and pLaS2 cDNA inserts. Both cDNAs are oriented 5' to 3' according to the mRNA sequence and are numbered so that the first base coding for the hormone somatostatin I or somatostatin II is no. 1, and with all bases in the 5' direction given negative numbers. Thickened areas of the line for each cDNA indicate the predicted translated region of the mRNA sequence and the double thickness at the 3' end of the translated sequence indicates the region coding for the hormone. Not included are the poly(dG)-poly(dC) tails at the ends of the cDNA inserts which were between 12 and 25 base pairs long. Below each sequence are arrowed lines indicating the restriction sites used for kinasing the DNA and the direction and extent of DNA sequence determined using that labelled fragment. To prepare purified fragments for each labelling or sequencing

reaction, DNA fragments were cut by the indicated restriction endonuclease, separated by polyacrylamide slab gel electrophoresis and electroeluted. All acrylamide gel electrophoreses were carried out in 100 mM Tris-borate pH 8.1, 2.5 mM NaEDTA using 5–7% polyacrylamide (20:1, acrylamide/bisacrylamide). To end-label, the DNA fragments were treated for 30 min at 65 °C with 8–20 units of bacterial alkaline phosphatase (Worthington) in 0.1 M Tris-HCl pH 7.4, 0.1 M NaCl, phenol extracted (2×), and then treated with 4–8 units of T4 polynucleotide kinase (BioLabs) at 37 °C for 30 min in a reaction mixture containing 50 mM Tris pH 7.6, 10 mM MgCl₂, 5 mM DTT, 200 μ Ci (3,000–4,000 Ci mmol⁻¹) [γ -³²P]ATP. The 5' end-labelled DNA fragment was subsequently cut by an intervening restriction endonuclease, separated by gel electrophoresis, eluted, concentrated and ethanol precipitated as described above. Sequence reactions were carried out according to Maxam and Gilbert⁴¹.



Two additional non-cross-hybridizing non-insulin cDNA inserts (later termed pLaS1 and pLaS2) were selected. The size of the mRNAs specific to both pLaS1 and pLaS2 were determined by 'Northern' gel transfer²⁴ analysis to be approximately 700 bases (see Fig. 1, lanes 3, 4). By a similar analysis, the other major poly(A)⁺ mRNA of 840 bases corresponds to preproinsulin (data not shown). The two non-insulin cDNA inserts were sequenced according to the strategy presented in Fig. 2. The nucleotide sequence of pLaS1 (563 base pairs) predicts the entire peptide sequence of preprosomatostatin I whereas pLaS2 (594 base pairs) predicts the sequence of preprosomatostatin II. The 14-amino acid sequence of the hormone in fish preprosomatostatin I is identical to mammalian somatostatin. This corroborates an earlier sequence analysis of fish somatostatin²⁵.

As expected from the lack of cross-hybridization and its restriction map (Fig. 2), the nucleotide sequence of pLaS1 insert DNA differs substantially from that of pLaS2; nevertheless, the molecules are clearly related. As shown in Fig. 3, the nucleic acid sequences were aligned based on the recognized homologies present in the regions coding for the hormone and the signal peptides. Additional homologies in the translated and in the untranslated regions were revealed by computer analysis. A molecule of approximately 45% overall homology can be constructed by the insertion of gaps in the nucleotide sequence. Beginning with the first AUG codon at the 5' end of the sense strand, translation of pLaS1 predicts the sequence of a 121-amino acid peptide which contains the somatostatin hormone at its carboxyl terminus (Fig. 3). This leaves 37 bases of untranslated sequence at the 5' end of the cDNA and 158 bases 3' to the stop codon and before the poly(A) tail. If translation is initiated at the first AUG codon (amino acid position -107), the predicted preprosomatostatin I peptide has a molecular weight of 13,328. This is approximately the size of somatostatin precursors which have been detected in both mammalian and fish tissue^{17,18,42}. The region of the peptide following this AUG comprises a sequence of hydrophobic amino acids characteristic of the signal peptide described for other precursors of secretory proteins²⁶. The sequence Leu-Leu-Val-Leu-Leu in position

-96 to -92 of this region is found in the signal peptide of fish preproinsulin and is located at approximately the same distance from the initiating methionine in both precursor molecules^{22,23}.

Translation from the first AUG codon in pLaS2 predicts a 125-amino acid peptide (MW 14,100) which also contains a characteristic signal peptide at its amino terminus and a 14-residue somatostatin-like peptide at its carboxyl terminus. This somatostatin II peptide differs from somatostatin I by two internal amino acids at position 7 (Tyr instead of Phe) and position 10 (Gly instead of Thr). The hormone sequences are flanked at their amino terminus by the residues Arg-Lys. Such contiguous basic residues are frequent sites for post-translational processing of hormone precursors^{27,28} and suggest that both somatostatins are cleaved from their precursors by a trypsin-like enzyme activity. There is additional homology extending to amino acid -18. This region of the anglerfish precursors is in turn homologous with a 28-amino acid somatostatin-containing peptide isolated from porcine gut²⁰ (see Fig. 4). The anglerfish somatostatins have arginine residues at position -15 and thus can both be cleaved by a trypsin-like activity to give similar 28-amino acid peptides. These conserved features of the molecules suggest that this 28-amino acid sequence may have biological activity. The additional 2-amino acid homology evident in the two fish precursors may indicate a recognition sequence for a processing enzyme. This same Leu-Glu-Arg sequence is found at a second position (-66/-64) in preprosomatostatin II.

Apart from the characteristic hydrophobic amino acid composition, there is little homology in the signal peptide region of the two preprosomatostatins.

The untranslated regions of pLaS1 and pLaS2 have some areas of sequence homology when aligned as shown in Fig. 3. However, it is not clear that these common sequences have a functional role. The homologies (>55%) in the cloned portion of the 5'-untranslated region are largely due to the repeated sequence XCAGAX. In the 3'-untranslated region, as in many other eukaryotic mRNA sequences, pLaS1 has a AAUAAA sequence beginning 20 bases from the polyadenylation site²⁸⁻³¹. This same sequence is present in pLaS2 but at a position 40

GAUCCGACAGCGCCGACAGUACAGCAUCACGUG
 * * * * *
 CAGAGACAAACCCAGCAGAACCAGAGAACACAGACACAGCAGACAGU:::
 -107 met lys met val ser ser arg leu arg cys leu leu val leu leu
 AUG AAG AUG GUC UCC UCC CGC CUC CUC CUC GUG CUC CUC
 *** ** * * * * *
 AUG CAG UGU AUC CGU UGU CCC GCC AUC UUG GCU CUC CUC GCG UUG GUU
 met gln cys ile arg cys pro ala ile leu ala leu leu ala leu val
 -111 -110 -100
 -90 leu ser leu thr ala ser ile ser cys ser phe ala gly gln arg asp
 CUC UCC CUC ACC GCC UCC AUC AGC UGC UUC UUC GCC GGA CAG AGA GAC
 *** * * * * *
 CUC UGC GGC CCA AGU GUU UCC UCC CAG CUC GAC AGA GAG CAG AGC GAC
 leu cys gly pro ser val ser gln leu asp arg glu gln ser asp
 -90 -80
 ser lys leu arg leu leu leu his arg tyr pro leu gln gly ser
 UCC AAA :: CUC CGC CUC CUC CAC CGG UAC CCG CUC CAG GGC UCC
 * * * * *
 AAC CAG GAC CUC GAC CUC GAG CUC CGU CAG CAC UGG CUC CUC GAG AGA
 asn gln asp leu asp leu glu leu arg gln his trp leu leu glu arg
 -70
 -60 lys gln asp met thr arg ser ala leu
 AAA :: :: :: :: :: :: :: CAG GAC AUG ACU CGC UCC GCC UUG
 *** * * * * *
 GCC CGG AGC GCC GGA CUC CUC UCC CAG GAG UGG AGU AAA CGG GCG GUG
 ala arg ser ala gly leu leu ser gln glu trp ser lys arg ala val
 -60 -50
 -50 ala glu leu leu leu ser asp leu leu gln gly glu asn glu ala leu
 GCC GAG CUC CUC CUC UCC GAC CUC CUC CAG GGG GAG AAC GAG GCU CUC
 *** * * * * *
 GAG GAG CUC CUC GCU CAG AUG UCU CUC CCA GAG GCC ACG UUC CAG CGG
 glu glu leu leu ala gln met ser leu pro glu ala thr phe gln arg
 -40
 -30 glu glu glu asn phe pro leu ala glu gly gly pro glu asp ala his
 GAG GAG GAG AAC UUC CCU CUC GCC GAA GGA GGA CCC GAG GAC GCC CAC
 *** * * * * *
 GAG GCG GAG GAC GCG UCC AUG GCA ACA GAA GGA CGG :: :: :: ::
 glu ala glu asp ala ser met ala thr glu gly arg
 -30 -20
 ala asp leu glu arg ala ala ser gly gly pro leu leu ala pro arg
 GCC GAC CUA GAG CGG GCC GCC AGC GGG GGG CCU CUC CUC GCC CCC CGG
 ** * * * * *
 AUG AAC CUA GAG CGG UCC GUG GAC UCU ACC AAC AAC CUA CCC CCU CGU
 met asn leu glu arg ser val asp ser thr asn asn leu pro pro arg
 -10
 Somatostatin I
 1 10
 glu arg lys ala gly cys lys asn phe phe trp lys thr phe thr ser
 GAG AGA AAG GCC GGC UGC AAG AAC UUC UUC UGG AAA ACC UUC ACC UCC
 *** * * * * *
 GAG CGU AAA GCU GGC UGU AAG AAC UUC UAU UGG AAG GGC UUC ACU UCC
 glu arg lys ala gly cys lys asn phe tyr trp lys gly phe thr ser
 1 10
 Somatostatin II
 14
 cys OP
 UGC UGA GCCGCUCCAUUCCGCGCUCCUCCGUCUCCACGAGCGUUUU:::ACAG
 ** * * * * *
 UGU UAA :: AGCUCCGCCAGCCAAAGCUACACCGUCACCGGACCAACCAUCCAGAUAC
 cys OC
 14
 ACGCUGAAUGGAUCCCGGUUUGCAGCUCCUCCUCCUUGGGCGGAGUCUGAAUGUAAACUUA
 ** * * * * *
 ACCUGAAAUUACCGUAGAAGACGACGACCAACAGCAGCUC::UCCGGAUGGAUGUACCC
 UGAAACUAAUUUUAAUGGUUGUUGAAUAAAAUUCUGUUGAGAG Poly A
 **** * * * * *
 UGAAUAAA:::UAAUACUGUUAUGAAUAAAGCGAUAAUCCGUC Poly A

Fig. 3 The mRNA nucleotide sequence and single-frame translation of pLaS1 and pLaS2. For comparative purposes, the two nucleic acid sequences are aligned (by the use of gaps) to show regions with a significant amount of base homology. The translated amino acid sequences are numbered by designating the first residue (Ala) of the somatostatin I and somatostatin II peptides as no. 1. The amino acids towards the amino terminus of the precursors are numbered negatively and those towards the carboxy terminus of the hormones are numbered positively. The asterisks indicate homologous bases along the pLaS1 and pLaS2 nucleotide sequence. We have, in addition, sequenced two other cDNA fragments which cross-hybridized with pLaS1 and two which cross-hybridized with pLaS2. In all three cases the sequences were identical to those presented here, eliminating the possibility of any significant errors resulting from the reactions involved in cDNA synthesis.

bases from the poly(A) site whereas a slightly different sequence, AAUUAUA, is found at the corresponding position closer to the 3' end of the mRNA.

Characteristics of somatostatin I and II precursors

Our results show that, in common with many other hormones, somatostatin is synthesized as part of a larger precursor peptide. This precursor contains a signal peptide which may be released during the transit into the endoplasmic reticulum; the resultant prosomatostatin would be approximately 97 residues for somatostatin I and 101 residues for somatostatin II (with MWs 10,600 and 11,600 respectively). The function of this form of the precursor, which is more than five times the size of somatostatin, is interesting. It may be cleaved in one step to yield somatostatin alone and thus have no function other than to act as a connecting peptide between the somatostatin hormone and the signal peptide. Alternatively, prosomatostatin, or a smaller, intermediate-sized precursor containing the somatostatin sequence, may be biologically active because 'big somatostatin' exhibits somatostatin activity¹⁹. Finally, there may be a related or separate biologically active peptide encoded in the precursor in analogy with the adrenocorticotropin (ACTH) precursor²⁸. However, a computer analysis of both preprosomatostatin I and II detected no distinct homologies with any known protein sequence (search completed by the National Biomedical Research Foundation, Georgetown University).

Two somatostatin activities?

The finding of two distinct mRNAs which generate different somatostatin molecules raises questions concerning possible functions of multiple somatostatins and the nature of somatostatin hormone activity measured in animal tissues by immunological procedures. The somatostatin I and II hormones are sufficiently closely related structurally for it to be likely that they cross-react to some degree in biological activity and/or radioimmunoassays. However, it is unclear whether they exhibit the same range of biological activities. Previous studies using chemically synthesized somatostatin analogues have shown selective inhibition of secretion of insulin or glucagon by the pancreas and/or growth hormone by the pituitary³². These studies indicate significant functional differences in somatostatin receptors of the different target cells. Therefore, it is possible that there are several somatostatins with common immunological determinants but with selective activities on different target cells. A test of the biological activity of somatostatin II as well as prosomatostatins I and II may help to resolve these questions.

We thank Dr John McCosker of the Steinhart Aquarium for help in obtaining fish specimens, Leslie Spector for assistance in

ANGLERFISH SOM. I LEU GLU ARG ALA ALA SER GLY GLY PRO LEU LEU ALA PRO ARG GLU ARG LYS ALA GLY CYS LYS ASN PHE PHE TRP LYS THR PHE THR SER CYS
 ANGLERFISH SOM. II LEU GLU ARG SER VAL ASP SER THR ASN ASN LEU PRO PRO ARG GLU ARG LYS ALA GLY CYS LYS ASN PHE TYR TRP LYS GLY PHE THR SER CYS
 PORCINE SOM-28 SER ALA ASN SER ASN PRO ALA MET ALA PRO ARG GLU ARG LYS ALA GLY CYS LYS ASN PHE PHE TRP LYS THR PHE THR SER CYS

Fig. 4 Alignment of porcine somatostatin-28 (ref. 20) to the corresponding peptide region of preprosomatostatin I and II. Boxed residues show homologies present in the precursor peptides; residues underlined within the hormone sequence indicate those that are unique to anglerfish somatostatin II.

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18,000 MW peptide synthesized *in vitro* by translation of anglerfish Brockmann body mRNA is immunoprecipitable using somatostatin specific antibody⁴³. A second report using the same method of analysis indicated that two peptides (16,000 and 14,000 MW) synthesized by anglerfish Brockmann body mRNA *in vitro* are immunoprecipitable using somatostatin specific antibody⁴⁴. The exact relationship between the peptides in these reports and the predicted peptides reported here is unclear.

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LETTERS

Observations of optical flares in the recurrent X-ray transient A0538–66

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The recurrent transient X-ray source A0538–66 probably lies at the distance of the Large Magellanic Cloud (LMC) and is consequently among the most luminous stellar sources known ($L_x \sim 8 \times 10^{38}$ erg s⁻¹, 2–17 keV) (refs 1–4). It has a unique combination of properties. The X-ray outbursts which have been observed were separated by multiples of a 16.6-day period. Some were relatively long, for example the $n = 3$ and $n = 4$ events (using the nomenclature of Johnston *et al.*²) lasted ~14 days and ~1 day respectively, but others lasted only a few hours. Observation of the $n = 3, 4, 11$ outbursts³ recently led to an improved error box and the proposal of a $B \sim 15$ optical counterpart⁴ (star 'Q' in Fig. 1 of ref. 4) shown on archival plates to be variable. The star was found to have an absorption spectrum characteristic of a B star. I report here an examination of 85 plates taken with the UK Schmidt telescope at Siding Spring, between 1974 and 1979 including some taken at the time of the X-ray observations. Dramatic flares observed on three of the plates, separated by intervals of 16 days and synchronized with the X-ray outbursts, confirm the proposed identification.

The region of sky containing A0538–66 has been included in numerous plates taken with the UK Schmidt telescope, both in its survey role and as part of non-survey programmes. The plates have been taken in various colours but in particular a series of 35 B plates overlapping the Ariel 5 and HEAO 1 X-ray observations have been measured with an iris photometer. The plates were taken largely on nights when conditions were not sufficiently good for survey work and are of rather variable quality. The plate-to-plate consistency obtained for a series of

comparison stars was 0.15 mag r.m.s. Excluding two plates, the r.m.s. scatter of the observations of the proposed candidate, star 'Q', about their mean was only slightly greater (0.20 mag) and this difference may be attributable to the influence of a $B \sim 17$ star 4 arcs away which is resolvable from Q on some, but not all, plates and which complicates the photometry. On each of the two remaining B plates Q was brighter by $\Delta B = 1.8$. Of these two plates one was taken during the 1-day long $n = 4$ X-ray outburst (17.1 UT on 4 September 1977) (Fig. 1a) and the other close to the time that the $n = 5$ X-ray outburst was expected (at 18.4 UT on 20 September 1977)³. Scans through the region made by the Large Area Sky Survey (LASS) instrument on HEAO 1 1.7 h before the latter optical observation and 4.7 h after it did not detect any X-ray activity (Fig. 1b).

The candidate star lies within the boundary of the stellar association NGC2034 and the surrounding region of the plates contains several stars of similar magnitude with which visual comparisons are easily made. This enabled a simple search to be made for optical flares on 50 further original plates and copies. The plates were of various colours but at least three of each type were compared (3U, 3B, 7V, 3R, 12I, 11J, 4H α , 7 non-standard). In most cases photographic enlargements of the region were used in the comparison. Changes of 0.5–1 mag would have been detectable, depending on the plate quality and colour. The flares of $\Delta B = 1.8$ are immediately apparent in this way.

The search led to the discovery of a third flare, on a plate taken using 098 emulsion and only a polaroid filter and therefore sensitive to a wide range of wavelengths from ~4,000 to 7,000 Å. The plate was taken during the $n = 6$ X-ray outburst, at 17.1 UT on 7 October 1977 (Fig. 1c). No detailed measurements have been made as the original plate is still in Australia, but comparison with copies of other plates taken with the same filter/emulsion combination suggests that the increase above the quiescent level is about the same as the 1.8 mag increase during the flares seen on B plates.

On eight plates taken between those on which the flares are seen the star was apparently in its normal state. These include two plates taken 23 and 24 h before that showing the third flare.

As there is no certainty that the optical luminosity was the same at the time of the 7 October plate as it was for the two *B* plates showing flares, there is no firm information on the colour in the flare state. The similarity of the three events suggests that the colour may not be very different from that in the quiescent state. Colour measurements based on the measurements of UK Schmidt plates are given in Table 1. Compared with the results of Johnston *et al.*⁴ these figures suggest the star is brighter (by 0.8 mag) and suggest a UV excess not apparent from their spectrum. Their results are based on plates taken in 1941–45, when the mean level may have been different, and on a spectrum obtained with the Anglo-Australian Telescope in July 1979, when the zenith distance was large, perhaps making the figures unreliable at shorter wavelengths (M. J. Ward, personal com-

Table 1 Photometry of the counterpart of A0538–66

	<i>B</i>	<i>U–B</i>	<i>B–V</i>	<i>V–R</i>	Broad band
Quiescent state	15.0	–1.5	0.1	0.6	
During flares	13.2				
Difference	1.8				~1.8

UVR measurements are from plates taken on the same night (10–11 h UT on 19 March 1979) presumably in the quiescent state. Colours are calculated assuming the mean *B* magnitude from 33 plates taken between November 1976 and January 1978 (not including those showing flares). Standards from refs 6–8 were used for calibration but none is close to star Q both in brightness and position and in addition to the effects of the internal scatter discussed in the text each measurement is subject to a calibration uncertainty of the order of 0.25 mag. Thus errors in colours could be as large as 0.4 mag.

munication). In addition the calibration uncertainties are large, as indicated in Table 1.

To estimate the optical luminosity we assume that the flares had the same spectrum as a *B*2.5 main sequence star, consistent with the quiescent-state spectrum discussed above. Then, assuming a distance modulus of 18.6 corresponding to the LMC and allowing for an extinction of 0.2, the *B* = 13.2 flare state corresponds to $M_{\text{bol}} = -7.6$ or an optical luminosity of 3×10^{38} erg s^{–1}. This is ~3 times higher than the X-ray flux measured at very nearly the same time as the *n* = 3 optical observation. If the spectrum is not that of a *B* star but is similar to the emission spectrum expected from an accretion disk⁵ then the optical luminosity would be higher still. Thermal bremsstrahlung from an optically thin plasma at 6.6 keV (ref. 1) capable of producing the observed X-ray flux would produce a *B* band flux ~250 times below that observed.

The star was observed by Johnston *et al.* in what can now be recognized as its flare state (although not as bright as those reported here) on a plate taken on 12 November 1945. Combining this with all other optical and X-ray observations (except the anomalously long and late *n* = 3, 4 events) and assuming a constant period plus a random phase jitter, best fits are obtained for periods of 16.668 ± 0.002 or 16.645 ± 0.002 days depending on whether the 1945 event is attributed to *n* = –693 or *n* = –594. The r.m.s. deviation is 1.5 h in either case. Other periods differing by steps of 0.023 days from these are possible but imply a jitter at least twice as large.

The three UK Schmidt telescope plates showing flares were among 26 obtained between September 1977 and March 1978 which have been examined. They occurred on consecutive cycles of the 16.6-day period. The fact that none of the 59 plates from other times during the period 1974–79 which have been examined shows any sign of flares suggests that the source may not always be active, although the X-ray activity lasted at least from June to December 1977 and there was previous optical activity in 1945. Plates taken near the times when outbursts would have been predicted on the basis of each of the above possible periods are among the 59 from other years which have been examined without detecting further activity. However, because of the uncertainty in the duration of the flares and of the existence of phase jitter, this evidence is inconclusive. The observation of the optical flare at *n* = 5 shows that either the X-ray outburst was very short or an optical flare occurred without a corresponding X-ray outburst.

Measurements of the plates to determine the position of the centroid of the emission in the flare state place it within 0.1 arc s of that in the quiescent state, effectively excluding the possibility of another, normally invisible star being the source of the events. Thus these results place beyond doubt the association of star Q with A0538–66, as proposed by Johnston *et al.*⁴

The discovery of the flaring optical counterpart considerably improves the chances of establishing whether there is a true constant period, perhaps a binary orbital period, controlling the activity of the system or whether a roughly constant characteristic time between outbursts produces a pseudo-periodic

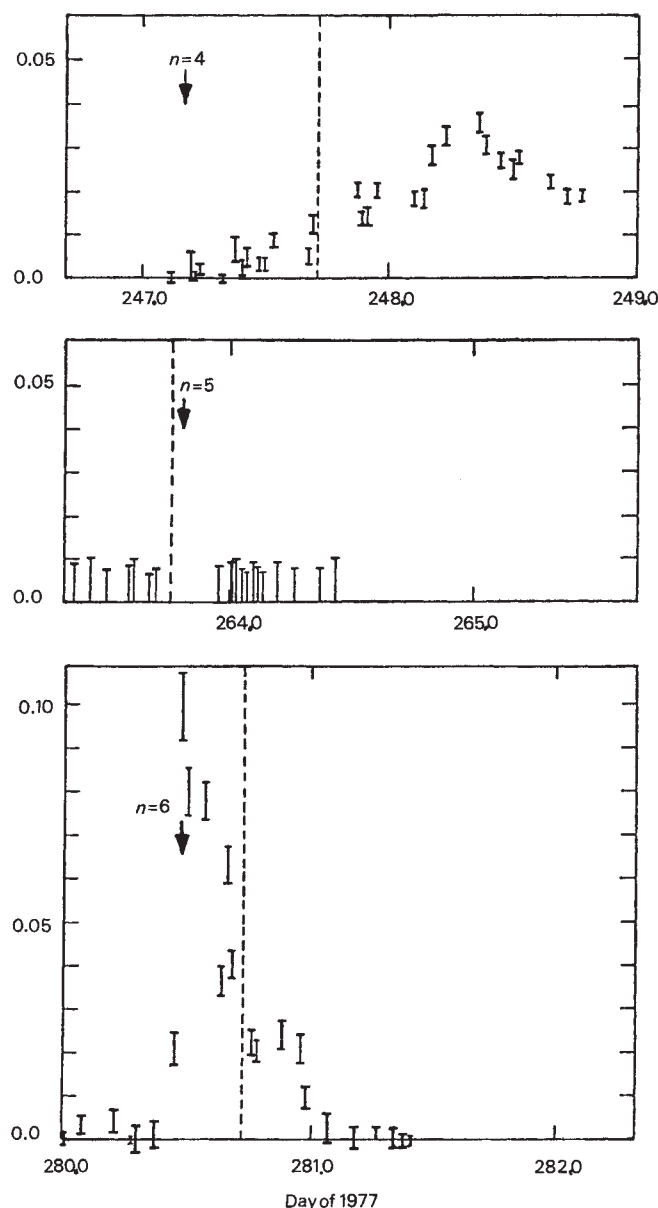


Fig. 1 The times of the three observations of optical flares (dotted lines) compared with the HEAO 1 LASS X-ray data. Plate exposures and X-ray measurement times are short compared with the ordinate scale (10–20 min; 5 s). The graphs are aligned and labelled according to the ephemeris given in ref. 4. In each case the ordinate shows X-ray intensities in units of the intensity of the Crab Nebula. The *n* = 4 X-ray event (a) is one of two anomalously long and late ones; the *n* = 6 event (c) is more typical. The points plotted in b are all 2σ upper limits. Not all available upper limits are shown but there were no observations during the gap around the time of the optical observation as the satellite was being used in pointed mode.

behaviour. The surprising absence of emission lines in the spectrum of the counterpart suggests that away from outburst the star is comparatively normal and the prospects of spectroscopic studies helping to unravel the nature of this unique system are consequently also good.

Regarding A0538-66 as an X-ray transient, its X-ray spectrum and the properties of the optical counterpart suggest that it should be classed with systems such as A0535+26, 4U0115+63 and A1118-61, which tend to have hard spectra and high mass counterparts, rather than the soft, low mass transients such as A0620-00, A1524-617 and 1H1705-250. Optical flares coincident with X-ray outbursts are common in the latter class (see review in ref. 9), but in these cases the optical flux is only a minor contribution to the overall luminosity during outburst. For example, although during the X-ray transient outburst of A0620-00 the optical counterpart increased in brightness by 3 mag, the optical luminosity was still less than the X-ray luminosity by a factor of 85. Much of the increase in optical flux in such cases is probably due to reprocessing of X rays in the atmosphere of a star whose quiescent state luminosity is low or in an accretion disk.

Similarly, optical bursts have been detected in coincidence with X-ray bursts from MXB1735-44 (refs 10, 11) and 4U1837+04 (ref. 12) but again the X-ray luminosity greatly exceeds the optical luminosity (by factors of 5×10^4 and 3×10^5 respectively) and the optical flux can be attributed to reprocessing.

Optical luminosities comparable with, or in excess of, the X-ray luminosities are known to occur in bright, high mass systems. Optical variability has been reported in several cases, but the changes have shown no correlation with X-ray outbursts. Nor have they been as large as those described here. Even if the distance of A0538-66 is regarded as uncertain, the relative increase in optical flux compared with the X-ray flux is unique. If the source is indeed at the distance of the LMC, then the absolute value of the increase is even more remarkable.

Many existing plates must contain records of the behaviour of A0538-66 and I would be pleased to liaise between astronomers having such material. A position and finding chart are given in ref. 4.

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Note added in proof: Since completing the above work I have identified a further flare on a blue plate from the Harvard plate stack. The plate was taken at Bloemfontein, South Africa, on 8-9 October 1932. The plate quality does not permit good measurements to be made but the increase seems to be similar to that on the UK Schmidt plate events. A plate taken 24 h earlier shows possible signs of some brightening but on others from the same year star Q seems to be similar to the 1974-79 quiescent state. Measurements of other plates from the collection confirm that the star was generally ~ 0.5 mag less bright in 1941-46. The 1932 event is consistent with a period of 16.667 days if a rather large jitter is assumed, but excludes the possibility of a 16.645-day period.

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Neutrinos and the age of the Universe

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The age of the Universe should be calculable by independent methods with similar results. Previous calculations^{1,2} using nucleochronometers, globular clusters and dynamical measurements coupled with Friedmann models and nucleosynthesis constraints have given different values of the age. We report here a consistent age, T_U , whose implications for the constituent mass density are very interesting and are affected by the existence of a third neutrino flavour, τ , and by allowing the possibility that neutrinos may have a non-zero rest mass.

Nucleocosmochronology places a lower limit on T_U of ≥ 8.7 Gyr essentially model independently³ as this value represents the mean age of the r -process elements. A model dependent T_U can be determined from a chemical evolution model of the Galaxy. The major uncertainty entailed is relating the mean age of the elements to the duration of nucleosynthesis. Such an analysis leads to a model dependent upper limit⁴ on T_U of ~ 20 Gyr. However, there are non-standard galactic evolution models which cannot be eliminated and which give ages greater than this.

Big-bang nucleosynthesis began with Gamow⁵ who recognized that an early hot universe would allow nuclear reactions to occur to build heavier elements. With the microwave radiation confirming the hot early universe it is obvious that the primordial abundances of the light elements— ^2H , ^3He , ^4He and ^7Li —become useful probes⁶. For example, the observed primordial ^4He , Y , leads to constraints on the number of light ($M_\nu \leq 1\text{ MeV}$) neutrino types^{7,8}. Detailed big-bang nucleosynthesis calculations relates Y to the present baryon density, ρ_B , which is at the very least a lower limit to the universe density, ρ . The exact relationship between Y and ρ_B is weakly dependent on the neutron half life and we have used τ_n of 10.61 min (ref. 9). The deuterium density decreases with increasing baryon density thereby setting a ρ_B upper bound from D/H lower bound. In addition, Y increases with increasing ρ_B , and the observational limit, $Y \leq 0.25$ and the fact that at least three neutrino flavours exist also sets an upper limit^{7,8} on ρ_B . The fact that both D and Y measurements argue against large ρ_B coupled with the fact that these arguments are rather insensitive to baryon density fluctuations^{10,11} further supports the big-bang model. Kazanas *et al.*² and Gott *et al.*¹ achieved their restrictive limits on T_U by equating the nucleosynthesis constrained ρ_B with the ρ implied by galactic dynamics. Here by allowing for non-zero mass neutrinos $\rho \geq \rho_B$ is possible. The resulting age constraints are somewhat relieved but are still significant.

Globular cluster ages, T_{GC} , independently determine T_U and it has been found that T_{GC} may be written in terms of the primordial stellar helium abundance and their metal abundances, Z . The result from stellar evolution models is¹²:

$$\log_{10} T_{GC} \approx 0.035 + 2.085(0.3 - Y) - 0.034(\log_{10} Z + 3) \quad (1)$$

where $10^{-3} \leq Z \leq 10^{-4}$ and T_{GC} is in 10 Gyr units. The range for Z , following Iben¹², encompasses metal poor clusters ($Z \sim 10^{-4}$) to clusters where turnoff ages indicating young stars are observed ($Z \geq 10^{-3}$). The Y uncertainty will be discussed elsewhere. Carney¹³ has recently calculated globular cluster ages by comparing effective temperatures of main sequence turnoff points with model isochron results and he has compared his results with the evolutionary isochrons of other workers. When these results are normalized to the proper Y and Z values they are in good agreement with equation (1). We assume a negligible time interval between the big bang and cluster formation.

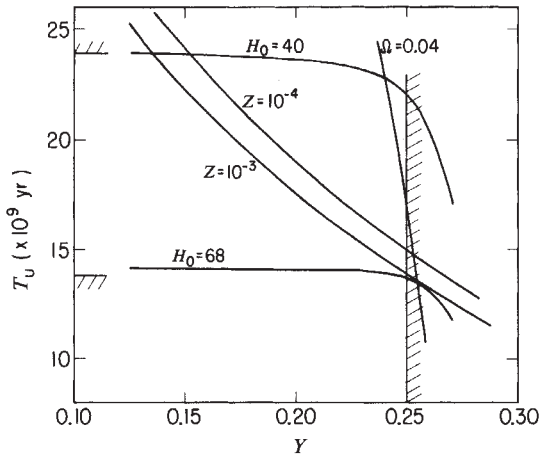


Fig. 1 The age of the Universe, T_U plotted against the primordial helium abundance, Y . The vertical line representing $Y = 0.25$ corresponds to the Y upper limit. The globular cluster ages are bracketed by their metal abundance, $10^{-4} \leq Z \leq 10^{-3}$. The constant density line, $\Omega = 0.04$, represents a lower limit to the total Universe density and $H_0 = 40$ ($\text{km s}^{-1} \text{Mpc}^{-1}$) represents the lower limit on the Hubble constant. The maximum allowed Hubble constant is found to be 68 ($\text{km s}^{-1} \text{Mpc}^{-1}$).

The third independent calculation of T_U involves a Friedmann model using zero cosmological constant ($\Lambda = 0$) representing a homogeneous isotropic universe presently matter dominated with negligible pressure. One can write:

$$T_U = F(\Omega)H_0^{-1} \quad (2)$$

where H_0 is the Hubble constant and $F(\Omega)$ is a monotonically decreasing function of Ω , where $\Omega = \rho/\rho_c = 8\pi\rho GH_0^{-2}/3$ (an exact description of $F(\Omega)$ is given in ref. 1). The density parameter, Ω , determines whether the Universe is open ($\Omega \leq 1$) or closed ($\Omega > 1$).

As pointed out by Kazanas *et al.*² precise values for the quantities necessary for T_U through the three methods—production and element abundance ratios for nucleocosmochronology, Y and Z for globular clusters, and H_0 and ρ (or Ω) for Friedmann calculations—would enable exact independent T_U determinations which could then be compared for consistency. Unfortunately none of the sets of quantities is known precisely and hence it is more useful to explore the range of the parameters that intersect T_U domains for a consistent age. A non-zero intersection will restrict T_U whereas a null intersection suggests a model flaw and forces one to question some of our basic astrophysical theories.

The calculation is most easily shown by plotting T_U against Y (Fig. 1). We expect a firm upper limit of Y to be 0.25 and a possible lower limit of 0.20. This range follows from Yang *et al.*^{7,8} who pointed that a Y value between 0.20 and 0.25 seems to be emerging from various experimental and theoretical determinations based on quite different input physics and assumptions. Recent measurements of population II subdwarfs indicating¹⁴ $Y \sim 0.19 \pm 0.04$ falls into this range within its uncertainty. However, it does cause concern in that the lower limit of 0.20 may be too restrictive.

From galaxy dynamics¹⁵ the probable limits for the total density parameter are $0.04 \leq \Omega \leq 0.4$ whereas the baryon lower bound is $\Omega_B \geq 0.001$ obtained from the local stellar neighbourhood¹⁶ or from the extremes of the mass implied for central regions of galaxies.

The previous calculation of Kazanas *et al.*² assumed a two neutrino standard big-bang model, that is the electron and muon neutrinos contributed to the radiation dominated early Universe density. The standard model now assumes three massless neutrinos because of the discovery of the τ lepton¹⁷. Even though the current experimental upper limit¹⁸ of the ν_τ mass is 250 MeV, which does not qualify as low mass for big-bang

nucleosynthesis calculations, the ν_τ is expected to be of low mass. Astrophysical constraints require it to be either very low mass and long lived or to have a mass ≥ 10 MeV and a lifetime $\leq 10^3$ s (refs 19, 20).

Figure 1 shows T_U plotted against Y for the standard 3ν model. If $\Omega = 0.04$ is an accurate lower limit and if it represents a baryon density then no concordant age is found. Even if 1 Gyr was allowed between the big bang and cluster formation no consistent age would be found. Thus if ν_τ is a low mass or massless neutrino then Ω_B must be less than the lower limit on Ω . This means that the bulk of the mass of the Universe would not be in the form of 'normal' baryonic matter. The most obvious alternative form is lepton matter. The recent reports of neutrino masses reported by Reines²¹ and the scales of neutrino clustering with galaxies are consistent with this²². In fact one could argue that this discrepancy almost requires that neutrinos have mass or that some other form of nonbaryonic matter exist.

It is also interesting that the maximum allowed Hubble constant is $68 \text{ km s}^{-1} \text{Mpc}^{-1}$. This conclusion is independent of the neutrino/baryonic mass ratio and derives only from the upper limit on Y and the globular cluster age consistency. This is true even in a lepton dominated universe as equation (2) requires $\Omega(\geq \Omega_B)$ and as a result for a given T_U , H_0 may only be lower than H_0 calculated for a baryon dominated universe because $F(\Omega) \leq F(\Omega_B)$. This H_0 upper limit conflicts with claims²³ of $H_0 \sim 100 \text{ km s}^{-1} \text{Mpc}^{-1}$. If H_0 were unequivocally shown to be large then the standard globular cluster age calculations and the assumption that the cosmological constant is zero would be questioned. However, the difficulties in direct measurements at H_0 suggest that there is no need to question such assumptions at present. It is probable that observationally $H_0 \geq 40 \text{ km s}^{-1} \text{Mpc}^{-1}$. This limit crosses the globular cluster ages at $\rho_B \sim 1.7 \times 10^{-32} \text{ g cm}^{-3}$ and $\Omega_B \sim 0.0053$ implying that $Y \geq 0.135$ and $T_U \leq 24$ Gyr. The aforementioned baryon density bound, $\Omega_B \geq 0.001$, is not very restrictive as such a constant density line would not intersect globular cluster ages until well above 25 Gyr. Likewise, the $\Omega < 0.4$ bound is not restrictive as $T \leq 0.25$ already requires $\Omega_B \leq 0.1$ (ref. 22). Likewise any Ω upper bound greater than 0.4 which may arise from lepton density considerations would not be restrictive.

The existence of a massive neutrino allows for a large lepton contribution to the Universe density and hence a lower baryon density (and lower Y) is possible. In fact, should neutrinos dominate the Universe density, which would be the case for a neutrino mass of a few electron volts²², then the T_U/Y relationship is broken as we have assumed Ω_B to be the dominant fraction of Ω in relating T_U to Y . Then, the aforementioned $T_U \leq 24$ Gyr would be the upper limit. We recognize that nucleocosmochronology coupled with a galactic evolution model set an upper bound of 20 Gyr and is more restrictive than this but we hesitate to use the 20 Gyr bound because of the uncertainties in the galactic evolution models. Significantly the 20 Gyr limit straddles the allowed range for Y and its credibility is also strengthened by the fact that the mean age of the elements normalized by the duration of nucleosynthesis has been found to be approximately constant for several galactic models⁴.

If the τ neutrino should be discovered to be massive (≥ 1 MeV) then the standard big-bang model should include only two neutrinos contributing to the early Universe density. The electron and muon neutrino model allows for a consistent age of 13.8–15.8 Gyr assuming $\Omega_B = \Omega \geq 0.04$. This restricts $0.237 \leq Y \leq 0.250$ and constrains the maximum Hubble constant to $64 \text{ km s}^{-1} \text{Mpc}^{-1}$. There would be no consistent age should $Y < 0.237$ or $\Omega_B > 0.18$. If we allow for a lepton dominated universe in this case as well and extend the lower bound to $Y > 0.13$ then one again finds $T_U \leq 24$ Gyr.

We have shown that there is no consistent age in the standard cosmological model if the τ neutrino has a mass < 1 MeV unless the density of the universe is significantly greater than the baryon density. Such a result argues for an earlier suggestion²² that neutrinos having masses of the order of tens of electron volts. Regardless of the mass of ν_τ and the mass of neutrinos in

general we re-emphasize that no consistent solution exists between standard globular cluster models and high values of the Hubble constant. If H_0 were shown observationally to be $\geq 70 \text{ km s}^{-1} \text{ Mpc}^{-1}$, then either standard globular cluster calculations must be seriously in error or else there must be a cosmological constant so that H_0^{-1} is no longer an upper limit for T_U .

Allowing for ρ to be $\gg \rho_B$ we find that the consistent age is

$$13.8 \text{ Gyr} \leq T_U \leq 24 \text{ Gyr}$$

If $\rho_B = \rho$ then consistent solutions are only possible if $M(\omega_\tau) > 1 \text{ MeV}$ which from ref. 18 would require $M(\nu_\tau) > 10 \text{ MeV}$ and $\tau(\nu_\tau) \leq 10^3 \text{ s}$. If these constraints are satisfied then the consistent age is similar to that of Kazanas *et al.*² and is,

$$13.8 \text{ Gyr} \leq T_U \leq 15.8 \text{ Gyr}$$

If neutrinos are shown to have electron volt masses then we are forced to conclude $\rho > \rho_B$ and the less restrictive age limits result.

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Brightening of a hot spot in NCG2903

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The nuclear region of the Sc galaxy NGC2903 exhibits a very chaotic structure with eight knots, described by Sandage² as “giant H II regions”. Others^{3,4} called these knots ‘hot spots’. This nuclear region has a very strong blue and UV emission^{5–7} and is a 1,420-MHz radio source⁸. Oka *et al.*¹ made photographic and spectroscopic observations of the central region, giving magnitudes and colour indices for the knots which they designated a–g. Physical conditions and stellar content of the 3-arc s central region were investigated by Alloin¹⁰ whereas Turnrose¹¹ analysed the stellar population in the knot c. The nucleus was identified on near IR photographs⁹. During high resolution—broad band and narrow band—photometric studies of the central region of NGC2903, we have observed and report here that one of the knots (a, according to Oka *et al.*¹) appears 1.3 mag brighter on plates taken in February 1980 than on a plate taken in January 1978, which agrees with other observations taken¹ in 1966–68.

Our observational material is described in Table 1. The 1978 plate was taken with the Griboval electrographic camera on the 30-inch reflector at the McDonald Observatory. Two other plates (167 P5 and 167 P6) were taken at Pic du Midi Observatory with the 20-mm Lallemand camera attached to the 106-cm reflector on 13 February 1980 as well as a test plate (167 P8) to correct for the S 20 photocathode inhomogeneities. On plates 167 P5 and 167 P6 the knot appeared brighter than on the McDonald plate by between 1 and 1.5 mag. On 18 March, knot a was observed visually with the camera eyepiece to be roughly as bright as the others, whereas in February the object was visually much brighter. The nuclear region as it appeared in January 1978 (McDonald plate with no filter) and in February 1980 (Pic du Midi plate 167 P6 with a V filter) is shown in Fig. 1. de Vaucouleurs has estimated from an electrographic B plate taken on 28 March, 1979 with the 76-cm McDonald reflector that the brightness of spot a was then about the same as in 1978.

The four plates mentioned above were scanned with the PDS microdensitometer at Nice Observatory. The field of the McDonald plate (15 arc min) allowed a reduction procedure following the numerical mapping technique¹² adapted to the interactive CDCA system¹³. The small field of the Lallemand plates (2.5×4 arc min) compared with the size of the galaxy did not allow such a procedure and only correction for the inhomogeneities of the photocathode was possible. Thus, the sky density was assumed to be constant and determined in one of the galaxy-density-free corner of the plates. Linearity between plate densities and light intensities were assumed on all plates after checking that densities were in the commonly adopted linear part of the characteristic curves of electrographic plates ($d < 3$). The zero point for the magnitudes was obtained from the integrated V magnitudes measured in various apertures by

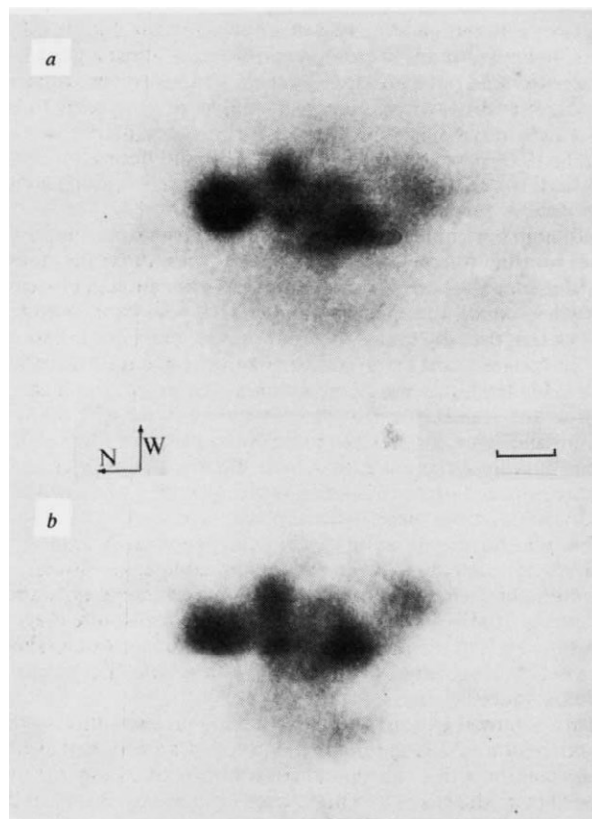


Fig. 1 Nuclear region of NGC2903. a, Pic du Midi plate taken with the Lallemand electrographic camera on 13 February 1980, with a V filter. b, McDonald plate taken with the Griboval electrographic camera on 31 January 1978 with no filter, a is the northernmost knot. Scale bar, 5 arc s.

Table 1 Observational material

Plate	Date	Camera	Telescope aperture (cm)	Emulsion	Filter	Exposure time (min)
GC1218	31 January 1978	Griboval	McDonald 76	Electro Image Film	No	30
167 P5	13 February 1980	Lallemand	Pic du Midi 106	Ilford G5	[O III]*	30
167 P6	13 February 1980	Lallemand	Pic du Midi 106	Ilford G5	V	10

* Centred on 5,000 Å with FWHM of 100 Å.

Table 2 Magnitudes and intensity ratios of the brightest knots

Knot	Okayama plates (1966–68)		Griboval plates (31 January 1978)		Pic du Midi V plate (13 February 1980)		Pic du Midi [O III] plate (13 February 1980)	
	Magnitudes V	Intensity ratio	Magnitudes	Intensity ratio	Magnitudes V	Intensity ratio	Magnitudes	Intensity ratio
a	14.2	1	14.0	1	12.7	1	—	1
c	14.3	~0.9	14.2	0.83	13.9	0.33	—	0.33
d	14.8	~0.6	15.0	0.40	14.6	0.18	—	0.18

photoelectric photometry, for plate 167 P6, taken with a V filter, as well as for the McDonald plate, taken with no filter. For the latter, this procedure does not lead to a strong error as most of the central intensity of the galaxy comes from the knots which have very similar colour indices ($B-V \sim 0.4-0.5$) (see ref. 1). Unfortunately for the [O III] plate, no absolute calibration can be derived. The derived V magnitudes may have errors of 0.1–0.2 mag. Note that Oka *et al.*'s V measurements for knots a and c correspond respectively to 5×6 arc s and 4×5 arc s elliptical regions whereas ours correspond respectively to 5×5 arc s and 4×4 arc s circular regions. As the variations of the sky density under the galaxy are not known for the Pic du Midi plates, the magnitudes presented in Table 2 are just a guide, so the variation of knot a is better perceived intensity-wise relative to knots c and d, whose luminosity might be supposed to be constant: knot a has become ~2.3 times brighter between January 1978 (possibly even March 1979) and February 1980. The [O III] plate corroborates this but cannot tell us much about the nature of this luminosity variation.

Although the knots have an emission spectrum superimposed on a continuum, measurements made with different detector/filter combinations are still comparable because, in one case at least (V band), the spectral bandwidth is very broad and the same as that used by Oka *et al.*¹. Moreover, even in the case of the McDonald plate (with no filter) and the Pic du Midi plate (with a V filter), the intercomparison keeps its full significance because the intensity ratio of knot c compared with knot d remains the same for the two plates (and even for Oka *et al.*), within the measurement errors, whereas the intensity ratio of knot a compared with c or d increases by a factor (~2.3) which is actually significant compared with these errors.

This brightening might be explained by supernova explosion occurring within knot a. From the data in Table 2, we estimate V magnitude of such a supernova at ~13.1. Assuming a distance modulus of 29.43 (distance 7.7 Mpc)¹⁴ gives an absolute magnitude of ~-16.5 after correction for absorption in our Galaxy ($A_v = 0.17$). This value is compatible with a type II supernova near maximum¹⁵.

Previous investigations^{1,10,11} have led to the description of the nuclear region of NGC2903 as the place of an active phase of star formation with a substantial contribution of young massive stars ionizing the gas. The knot a itself because of its off-centre position was missed in all relevant observations, but we may assume that these considerations are also valid for knot a as well as for hot spot nuclei in general^{9-11,16-20}. Therefore, the explosion of a supernova would not be surprising in such a region. Only a few examples are known of supernova phenomena in central regions of galaxies (for example, SN1885 in M31, SN1968 in M83 (ref. 21)). This might be due to

observational bias because an exploding object is harder to detect in a high-surface brightness region, but is probably more frequent than believed. We therefore suggest routine observations of such hot spots to investigate whether such brightenings are the result of supernova outbursts or hot spots are intrinsically variable objects. We hope that other observations of the nuclear region of NGC2903 in 1979–80 will be found to reconstruct the history of this event.

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A nonrandom component in cosmic rays of energy $\geq 10^{14}$ eV

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We have been studying¹ the distribution of the arrival times of atmospheric Cerenkov light pulses, initiated by cosmic rays of energy $\geq 10^{14}$ eV. We have now detected a nonrandom component for time separations < 40 s, and here we discuss the possibility that it has a point-source origin.

The light pulses were detected at Gulmarg (altitude 2,743 m), India, on clear moonless nights, by two, coincidentally operating wide-angle photomultipliers (EMI 9545 B; viewing angle with respect to zenith, 50°). Each Cerenkov light pulse, identified by its characteristic shape, was recorded with its occurrence time by a logic system with a dead-time of ≤ 100 ms. The amplitude discrimination levels of both the detector channels were held constant above the shot-noise fluctuations in the night-sky background light. The detection system was regularly checked with a standard light-pulsar to ensure that the photomultiplier and amplifier gains remained unaltered. Between June 1976 and June 1978 observations were possible for 180 h and 9,879 events were recorded, corresponding to an average rate of ~ 55 events per h. The observed hourly rates show a fairly symmetrical scatter around this value.

Using all the data, we have plotted the arrival-time distribution of the events, that is, the distribution of time-separation t between consecutive events on a 10-s bin size (Fig. 1). The smooth curve shows the expected exponential distribution of the arrival times assuming that the time of occurrence of the events is completely random; the inset to Fig. 1 represents standard deviations of the frequencies of the events with respect to the fitted curve. Deviations at $\geq 5\sigma$ are apparent for $t \leq 40$ s, the implied overabundance constituting $\sim 12\%$ of the total number of events.

The possibility that this overabundance was of instrumental origin was examined by subjecting the detection system to a random time-series simulation, in which the system photomultipliers were simultaneously exposed to light pulses from a pair

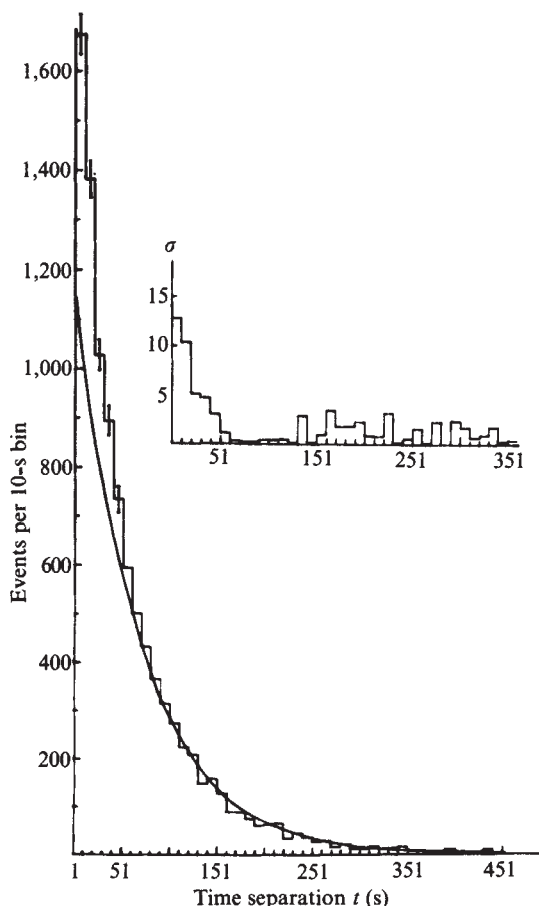


Fig. 1 The histogram shows the observed distribution of time separations, t , between consecutive events on a 10-s bin-size. Error bars up to $t = 50$ s are also shown. The smooth curve is the exponential fit to this histogram. Standard deviations, σ , of the event frequencies with respect to the fitted curve are shown in the inset.

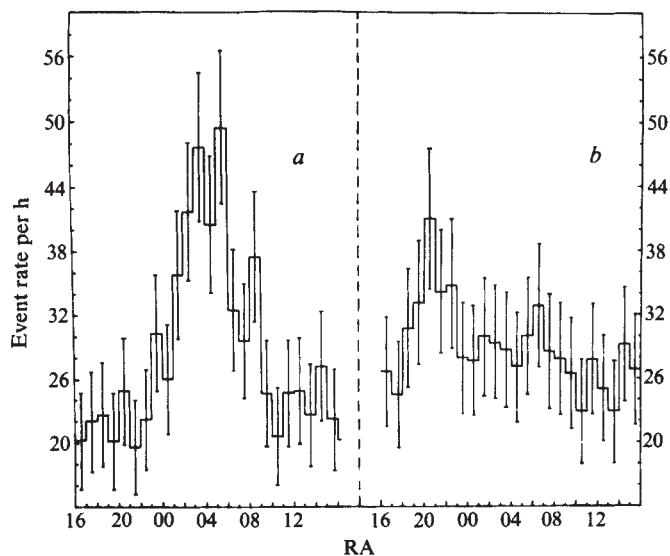


Fig. 2 *a* Shows the rate per h of events as a function of the RA values. Events with $t \leq 40$ s only are included. *b*, Same as *a* except that events with time separations of 41–450 s alone are included in the distribution.

of LED lamps. The flashing rate of the lamps was so controlled by a random pulse generator that pulses with time separations of 1–500 s were possible. The output from the detection system in this case was found to reproduce quite accurately the expected exponential variation in time. Likewise, solar-terrestrial origin of the overabundance also seems improbable because the event rates did not exhibit systematic solar time variations and also because the arrival-time distributions for days of high and low values of the geomagnetic disturbance index A_p , when considered separately, reproduced the excess contribution for $t \leq 40$ s to the same extent. Clearly, also, at these energies the effect cannot be understood in terms of the interplanetary magnetic field fluctuations. Furthermore, Baadino *et al.*², following our earlier work¹, have also observed a significant extra contribution for $t \leq 37.5$ s in muons produced by cosmic-ray primaries of energy $\sim 5 \times 10^{13}$ eV. Therefore, the observed excess is not peculiar to the optical method of detecting high-energy cosmic rays.

To find whether the observed excess has a sidereal association, the rates of two subsamples with $t \leq 40$ s and $40 < t \leq 450$ s, constituting 5,033 and 4,846 events, respectively, were plotted (Fig. 2) as a function of right ascension (RA), the RA value of an event being derived from its absolute occurrence time. It is clear that, whereas events with $t > 40$ s do not show a directional preference (Fig. 2*b*), those with $t \leq 40$ s, constituting the excess region, exhibit a statistically significant peak at $RA = 05 \pm 03$ h (Fig. 2*a*). To investigate this association further, events with $t \leq 40$ s were divided into two 1-year subsamples, and the directional enhancement at $RA = 05 \pm 03$ h was observed in each of them separately. The large width of the peak in Fig. 2*a* is compatible with the response function of our wide-angle detection system for Cerenkov light from EAS³. The large angular response of the detection system was also responsible for our observation of a significant excess of events for $t \leq 40$ s even for the period December 1978–January 1979, when, during a search for optical bursts from primordial black-hole explosions^{1,4}, a similar experiment was carried out simultaneously at Gulmarg and 30 km away at Srinagar (altitude 1,524 m). Figure 3 shows the sidereal distribution of the event rates for $t \leq 40$ s for the two data samples obtained from this experiment. It is apparent that the rates in both the samples show a clear rising trend beyond 22 h RA, which, if data beyond January 1979 were available, would probably reproduce the peak observed in Fig. 2*a* and so provide additional confirmation

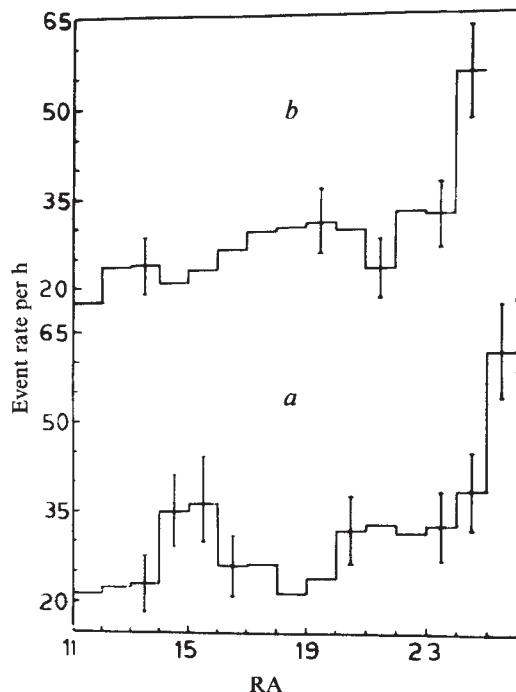


Fig. 3 Hourly rates of events with $t \leq 40$ s as a function of RA for December 1978–January 1979. Data recorded at Gulmarg (a) and Srinagar (b).

regarding the sidereal association of the excess. In view of this, it is useful to determine whether this excess contribution is due to a high flux of $\geq 10^{14}$ eV charged particles or to γ rays coming presumably from a cosmic source with $RA = 05 \pm 03$ h.

For a cosmic-ray proton of energy $\geq 10^{14}$ eV, the gyro-radius in the galactic magnetic field is ≥ 0.03 pc, which is quite small relative to the typical scale-lengths of the interstellar magnetic field irregularities, generally believed to be ~ 1 –100 pc (ref. 5). According to the compound diffusion model⁶, a cosmic-ray proton follows the field lines for distances less than 1–100 pc and is then transferred to other field lines in a random manner. Accordingly, charged particles coming from a galactic source, a

few kpc away, suffer scattering in the interstellar space and are not, therefore, expected to retain original source direction. On the contrary, one may, following Barrowes⁷, hypothesize that there are very few small-scale irregularities in the interstellar magnetic field in the direction of the source, so that charged primaries, gyrating around a quite well ordered magnetic field, reach us without undergoing sufficient randomization. The observed directional enhancement is, however, more easily understood if one assumes that the source is emitting γ -ray photons of energy $\geq 10^{14}$ eV, which the present detection system cannot distinguish from charged primaries. Several instances of discrete source anisotropies have been reported^{8,9} including the point sources at $RA = 6$ h, dec. $\delta = 6^\circ \pm 1^\circ$ and at $RA = 5$ h, $\delta = 0.5^\circ$.

If this view of the point-source origin of the excess contribution is valid, its time-structure will probably provide further information on the nature of the source. Accordingly, we plotted (Fig. 4a) the arrival-time distribution of events with RA values between 22 and 9 h only, which correspond to the abscissa limits of the peak in Fig. 2a. The distribution of the arrival times of the excess events, plotted in Fig. 4b, shows a significant peak at ~ 4 s, suggesting that there is a departure from time-randomness and that in the case of source events, $t \sim 4$ s is more probable than smaller arrival times, conceivably a manifestation of some kind of modulation due to the source. Extensive theoretical work by Ostriker and Gunn¹⁰ and others shows that indeed a rotating neutron star is capable of producing cosmic rays of energy $\geq 10^{14}$ eV. In view of this and the fact that the excess contribution peaks at ~ 4 s, it is tempting to infer that the source may be the pulsar PSR 0525 + 21, which has a period of 3.7 s and lies at a distance of 1.6 kpc (ref. 11). If so, it may be argued that high-energy particles, probably γ rays, are emitted randomly in time from a restricted region on or near this pulsar. The effect of the pulsar rotation period of 3.7 s would be seen by an Earth-bound observer as an enhanced frequency for t near 3.7 s and its harmonics relative to the other arrival times, which would be reduced in frequencies depending on the angle of the emission cone. Obviously, the event rate from the source cannot depend on the pulsar rotation period alone but also depends on distance, intrinsic flux and other emission characteristics of the source.

One may surmise, alternatively, that intense particle bursts are emitted towards the Earth with the pulsar period of 3.7 s. As the observed average interval between consecutive events is ~ 1 min, that is, nearly 16 pulsar periods, one would rule this out

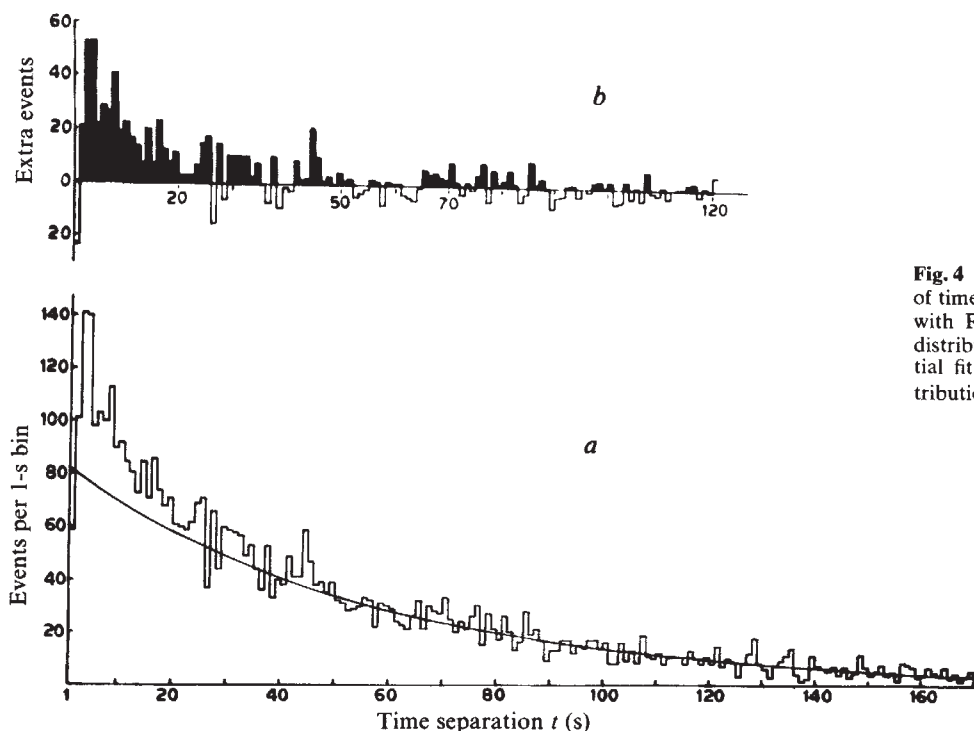


Fig. 4 a, The histogram shows the distribution of time separations, t , on a 1-s bin size. Events with RA values 22–9 h are included in this distribution. The smooth curve is the exponential fit to this distribution. b Shows the distribution of extra events obtained from a as a function of t .

unless an additional restriction is imposed that the bursting activity lasts only for a fraction of the total observing time. Further experiments in the Northern Hemisphere by air shower arrays and optical detection systems, designed with improved angular resolution and a bias towards photon-initiated showers, would be helpful in this connection.

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Extremely relativistic electron-positron twin-jets form extragalactic radio sources

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The extended extragalactic double radio sources—quasars, BL Lac-type-objects (blazars), and radio galaxies—are commonly interpreted as synchrotron sources in which extremely relativistic electrons gyrate in enhanced magnetic fields. The detection of narrow emission bridges between hot spots inside these sources and the centre of an intermediate galaxy has strongly favoured the existence of a continuous power-line feeding the extended source^{1,3,5}. Such beams may be a universal phenomenon occurring in many—if not all—massive galaxies, including our own⁴. But whereas early interpretations involved low-frequency electromagnetic waves and/or relativistic particle beams, more recent work favoured non-relativistic ($\beta < 10^{-1}$) (refs 3, 6–8), or mildly relativistic ($\gamma < 10$) (refs 9–11) bulk velocities for the power supply. In particular, non-relativistic bulk velocities $c\beta$ have been derived from estimates of the involved kinetic energy densities u and mass densities ρ in the form $\beta \approx (2u/\rho)^{1/2}$. This can lead to large underestimates of β when ρ is derived from Faraday rotation and depolarization data, because the observed jets are likely to have a two-fluid structure, with light relativistic plasma streaming inside of heavy ‘walls’ of thermal matter, or traversing ‘swarms’ of heavy quasistatic filaments¹². We suggest here that these beams consist of extremely relativistic electrons and positrons, of typical Lorentz factor $\gamma \geq 10^2$.

The power streaming in the jets must be liberated inside the galactic nucleus, from matter spiralling into the central power-house. This power $\dot{E}_{\text{lobes}}$ should be a small fraction η ($\ll 1$) of the total liberated power $\dot{E}_{\text{tot}}$ because of its comparatively low entropy. The power $\dot{E}_{\text{tot}}$ in turn is a small fraction ϵ (< 1) of the accreted mass–energy rate $\dot{M}_{\text{in}}c^2$, where $\epsilon \leq 0.1$ is suggested by black-hole models whereas hydrogen burning¹³ (of $\leq 20\%$ of the fuel) amounts to $\epsilon \leq 2 \times 10^{-3}$. The rest mass rate $\dot{M}_{\text{lobes}} = \dot{E}_{\text{lobes}}/(\gamma - 1)c^2$ injected into the lobes cannot exceed the fuel-

ling rate $\dot{M}_{\text{in}}$, hence

$$1 < \dot{M}_{\text{in}}/\dot{M}_{\text{lobes}} = (\gamma - 1)/\epsilon\eta \quad (1)$$

or $\gamma > 1 + \epsilon\eta$; which would imply, for non-relativistic values of $\beta = (1 - \gamma^{-2})^{-1/2}$:

$$\beta > (2\epsilon\eta)^{1/2} = 1.4 \times 10^{-2}(\epsilon\eta/10^{-4})^{1/2} \quad (2)$$

that is slow bulk speeds $c\beta$ of the beam material imply a small ‘beaming efficiency’ η . But η is likely to be above 10^{-2} . To see this, note that $\dot{E}_{\text{lobes}}$ can exceed the luminosity L_{lobes} of the lobes when most of the beam energy is stored in relativistic gyrations, and that a substantial fraction of L_{lobes} can be emitted near optical frequencies¹⁴, whereas the compact nuclear source is expected to emit its excess energy instantaneously as high surface-brightness electromagnetic radiation, mainly between IR and γ -ray energies. We thus have

$$\eta = \dot{E}_{\text{lobes}}/\dot{E}_{\text{tot}} \geq L_{\text{lobes}}/L_{\text{core}} = 10^{-2\pm 2} \quad (3)$$

the latter for well-studied sources like 3C273, Cen A, Cyg A, and from source statistics. Equation (2) then implies beam particle velocities in excess of 10^9 cm s^{-1} .

A second estimate of β derives from the speed at which the lobes of double sources separate. This speed has been estimated from the core–hot spot separations^{15,16} to be of the order of $v_r = 10^{-1\pm 0.3}c$. Similar values for v_r can be obtained from force balance calculations¹². The flow inside the jets must reach the outer ends of the radio lobes, hence its velocity must exceed v_r .

The flow inside the (almost free) jets is expected to be supersonic, although it must go subsonic at its termination point. Consequently, v_r is even a lower limit on the sound speed of the hot-spot material, whose temperature T_s must therefore exceed $m v_r^2/3k \geq 10^{10} \text{ K}(m/10^{-24} \text{ g})$. Were the jets subsonic, T_s would at the same time be a lower limit to the jet temperature. For a supersonic jet, on the other hand, there must be an inner shock surface towards the ambient medium where the flow passes from supersonic to subsonic. One can then relate the post-shock temperature T_s^+ to the pre-shock temperature T_s^- and get a more severe lower limit to the jet velocity.

If we assume that the jet velocity were sub-relativistic, its pre-shock sound speed v_s^- would be related to its post-shock sound speed v_s^+ by¹⁷

$$v_s^-/v_s^+ \approx [(\Gamma + 1)/(\Gamma - 1)]^{1/2}(p^-/p^+)^{1/2} \quad (4)$$

where $p^\pm$ are the pressures before and behind the shock respectively, and Γ is the adiabatic index. The pressure ratio p^+/p^- is unlikely to exceed 30; it can be estimated from the brightness ratio at different parts of the hot spot: In a few cases, a scintillating component has been found inside the hot spot whose inferred pressure is about 10 times higher than that of its surroundings^{18,19}. For $\Gamma \leq 5/3$, equation (4) therefore yields $v_s^-/v_s^+ \geq 0.6$. But v_s^+ must exceed the post-shock flow velocity v^+ which is larger than the lobe velocity v_r , hence $v_s^- \geq 0.6 v_r \geq 0.06 c$, corresponding again to a pre-shock temperature $T_s^- \geq 10^{10} \text{ K}(m/10^{-24} \text{ g})$. This lower limit conflicts with independent temperature estimates³ ($T_s^- \leq 10^7 \text{ K}$) of the jet material, that is excludes non-relativistic jet velocities. Also, adiabatic cooling in the jet would imply extremely relativistic temperatures near the origin.

Relativistic bulk velocities of the beam material are also suggested by the following independent observations whose details are discussed elsewhere¹²:

(1) Roughly half of the strongest compact extragalactic radio sources show intermittent superluminal expansion, on scales between 10 and 10^2 light yr, with apparent expansion speeds ranging up to $40 c$ (for 3C454.3)^{20,21}. These speeds must, of course, be phase velocities, but all reasonable models involve relativistic bulk velocities²² of the beam material.

(2) In most cases, the observed jets are one-sided, both in radio, optical, and X rays. Even in sources with a large inclination angle of their axis, like Cyg A, the VLBI structure is one-sided²¹. Relativistic beaming is the only convincing explanation¹².

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(3) The largest extragalactic radio sources²³ have unprojected tails of length $r \geq 3 \times 10^7$ light yr, and their central engines are still active. A non-relativistic power supply would make the central engine very old, and its fuel demand very high.

(4) When a beam of magnetized plasma is blown into some ambient plasma, its hot-spot develops at the far end of the resulting jet only if its mass density is much lower than that of the surroundings. In the opposite case, the hot-spot develops at the near end. But a lower mass density implies a higher sound speed (than some 10^8 cm s⁻¹), and an even higher (supersonic) bulk velocity.

(5) The beams of most sources are bent, in particular those of the head-tail galaxies. The standard explanation of bent beams uses ram pressure confinement^{5,7}, in which case the bending angle is proportional to the bulk velocity of the power supply (for a given power). Non-relativistic supply velocities would in some cases ask for implausibly high ram pressures of the intergalactic medium.

(6) For non-relativistic power supply velocities, all the relativistic electrons in the jets and lobes would have to be accelerated *in situ*. According to the second law of thermodynamics, this is only possible for a small fraction of the total power.

(7) The observed stability of the (often curved!) jets, on length scales exceeding 10^5 light yr, poses problems for non-relativistic bulk velocities¹².

We shall now suggest that the beams consist of electrons and positrons, with ions being energetically insignificant. Consider if there were no positrons, and n_e was the particle number density of the electrons, n_i the corresponding density of the (positive) ions. Charge neutrality in the beam implies $n_e = n_i$. The ratio ξ of energy densities in ions and electrons then reads

$$\xi = n_i \gamma_i m_i c^2 / n_e \gamma_e m_e c^2 \geq 1.836 \gamma_i / \gamma_e \quad (5)$$

where γ_i , γ_e are the average Lorentz factors of ions and electrons respectively. ξ should not exceed unity by much because the power stored in ions is invisible, and the lobe power $\dot{E}_{\text{lobes}}$ inferred from the synchrotron radiation of the electrons ought to stay significantly below the core power $\dot{E}_{\text{core}}$ not to violate the second law of thermodynamics, see equation (3). But then equation (5) implies large Lorentz factors for the electrons: $\gamma_e \geq 2 \times 10^3 \gamma_i$, larger than inferred from equipartition estimates for the energetic peak of the electron distribution. In other words, if the charge of the electrons were neutralized by the charge of ions, the necessary inequality $1 \gg \eta = \dot{E}_{\text{lobes}} / \dot{E}_{\text{core}}$ would be violated, either by a power excess in the ions, or by a significant deviation from equipartition (between magnetic and electron kinetic energy).

This argument can be strengthened by interpreting the frequent and pronounced one-sidedness of the jets as being due to extreme bulk velocities ($\gamma_{\text{bulk}} \geq 10^2$) of the electrons radiating when their path is obstructed¹², rather than as being due to mildly relativistic motion at a small beam inclination angle: The bulk velocities of oppositely charged particles cannot differ by more than $10^{-11} c$ ($\dot{E}_{\text{lobes}} / 10^{44} \text{ erg s}^{-1}$)^{1/2} because their relative motion would otherwise generate higher than equipartition magnetic fields. Consequently, the bulk Lorentz factor of the electrons must equal γ_i , and the condition $\gamma_e \geq 2 \times 10^3 \gamma_i$ would imply $\gamma_e \geq 2 \times 10^5$, that is extreme deviation from equipartition.

There are other indications of the near-absence of ions in the beams¹²; briefly they are:

(1) The direct supply of extremely relativistic electrons to the lobes avoids the difficulty of upgrading thermal energy of a mildly relativistic plasma to much higher energies of most of the electrons.

(2) No booster of two relativistic electron-ion plasma beams is known; the situation is different for an electron-positron plasma wave-riding a strong spherical wave, and focused by a magnetic pinch¹³.

(3) The smallness of observed Faraday rotation in compact sources rules out the presence of a significant fraction of low-

energy electrons, but does not rule out a neutral electron-positron plasma²⁷.

(4) It has been suggested that the relativistic wind of the Crab pulsar consists of an extremely relativistic electron-positron plasma^{24,25}. Galactic centre sources are likely to follow a similar working scheme¹³.

(5) The observed positron annihilation radiation from our galactic centre²⁶ is reported to exceed that likely to be injected by young pulsars by roughly two orders of magnitude. An active past of our galactic centre would yield a plausible interpretation.

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Flux tube dynamo approach to the solar cycle

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In the past two decades dynamo models of solar activity and the physical foundations of the solar cycle have become acceptable. However, recent observations revealing the concentrated form of photospheric magnetic flux¹, the distribution and cyclic appearance of X-ray bright points^{2,3} and ephemeral active regions⁴, as well as the discovery of sunspot brightness variations during the solar cycle^{5,6}, have raised some questions about the dynamo theory of solar activity⁷. Both observation and theory⁸ suggest that most of the magnetic flux in the solar convection zone is in the form of concentrated isolated magnetic flux tubes. The turbulent dynamo theory^{9,10} has, therefore, to be modified and attempts have been made to include concentrated fields¹¹. Nevertheless, the dynamo problem for a convective medium pervaded by concentrated flux tubes, the 'flux tube dynamo', has not yet been solved. The problem is to regenerate the poloidal magnetic field out of which toroidal field is produced by differential rotation. Here the consequences and the structure of the resulting cycle are considered if a field regeneration process operating on flux tubes is assumed. A calculation similar to Leighton's magneto-kinematic model¹² shows that a flux tube dynamo model can operate and reproduce the essential features of the solar cycle. It can also explain the cyclic variation of sunspot brightness and ephemeral active regions.

I conjecture that the variation of sunspot brightness and appearance of ephemeral active regions (EAR) or X-ray bright points (XBP) is caused by different age of the basic flux tubes. If EAR are interpreted as products of 'shredding' of big flux tubes by violent convective motions, older flux tubes will suffer longer

from this influence than younger tubes. Consequently, they yield more EAR and some may be totally disrupted before they reach the photosphere. The effect of age on sunspot brightness is not as obvious because of the lack of a detailed theory of the deep structure and cooling of sunspots. As sunspots are already cool when they appear in the photosphere, the cooling 'machine' must work beneath it. A plausible mechanism is the formation of loops with subsequent adiabatic downflow within the solar convection zone through a convective instability. If this happens at the beginning of the tube's existence it will subsequently be heated by radiation (and possibly convection of reduced efficiency because of the magnetic field). The older a flux tube, the longer the time available for heat exchange: emerged older tubes will appear brighter than younger ones. If the convective instability happens in the upper (more superadiabatic) layers of the convection zone, the initial conditions depend on flux tube age: older tubes are more severely distorted and 'shredded' by convective motions and possibly the instability will not be as efficient in cooling as for younger tubes. Finally, distorted old tubes might produce more umbral dots after emerging as sunspots, leading to a brighter appearance of the umbra when observed with normal resolution.

As long as the subphotospheric cooling of magnetic flux is not well understood the above arguments are speculative. Nevertheless, convection causes old flux tubes to be physically different from young tubes and the above considerations suggest that they are less coherent and not as cool as younger tubes.

Age differences between flux tubes can arise as follows. Consider a shear region of strong differential rotation where toroidal flux is produced out of a poloidal magnetic field. The flux tubes built by this process leave the shear region due to their magnetic buoyancy depending on the magnetic fields strength: stronger fields are more buoyant and rise faster. The stronger the poloidal field, the faster the toroidal field increases and the faster (and younger) the flux tube leaves the shear region¹³. If the poloidal field strength varies during the cycle, the age of the flux tubes which appear in the photosphere also shows a cyclic variation.

To study the value of the age hypothesis and to get some insight into the behaviour of a flux tube dynamo I have investigated a simple one-dimensional model.

Consider a sphere with a thin shear region due to differential rotation $\partial\Omega/\partial r$, idealized by a shell at $r = r_1$. In the axisymmetric case, the magnetic field in the shell only depends on the colatitude $0 \leq x \leq \pi$ and time t .

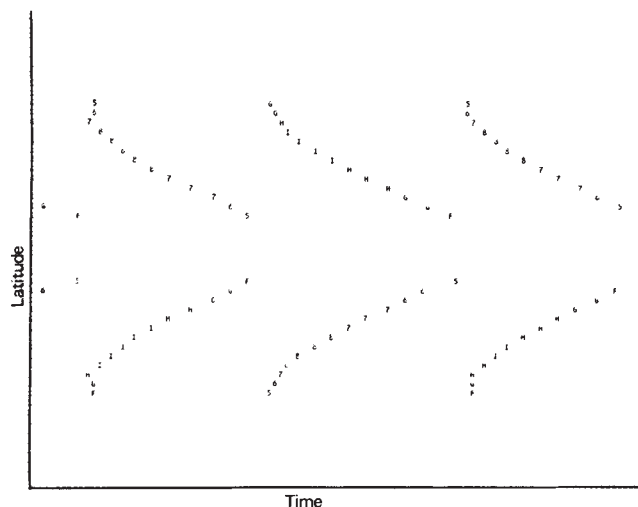


Fig. 1 A synthetic butterfly diagram. The latitude of appearance of flux tubes is plotted against time. Their activity lasts for months and leads to the broad wings of observed butterfly diagrams. Tubes appearing in high latitudes are weak and old: they are probably disrupted totally and do not build big active regions with spots.

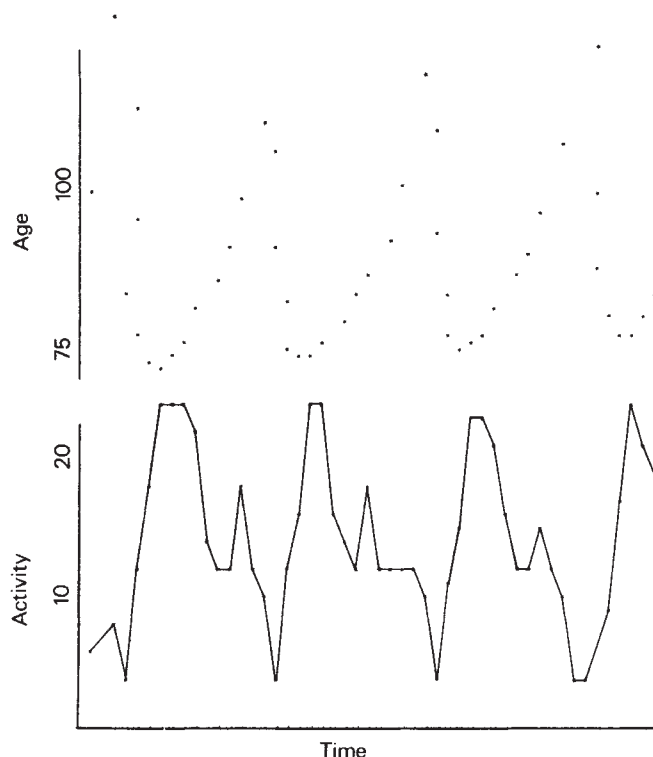


Fig. 2 Activity index and flux tube age: oldest flux tubes appear during activity minimum and vice versa. The age as a function of time shows a striking similarity to the variation of sunspot brightness and the number of XBP in time^{5,6}.

The toroidal field is determined by the ϕ -component of the induction equation at $r = r_1$:

$$\frac{\partial B_\phi}{\partial t} = B_r \cdot \sin(x) \cdot r_1 \cdot \left(\frac{\partial \Omega}{\partial r} \right)_{r=r_1} \quad (1)$$

We define a critical field strength, B_c , comparable with the equipartition with the convective kinetic energy. For $B_\phi \geq B_c$ convection is suppressed within the flux tube, the tube has an 'identity' and we start to count the age, A . The tube tends to leave the shear region because of its buoyancy with a probability assumed to depend on the product of field strength and age, $B_\phi \cdot A$. We allow for a random component in the cycle by defining the eruption probability P in the following way, using constants C_1, C_2 :

$$0 \leq B_\phi \cdot A \leq C_1: P = 0$$

$$C_1 \leq B_\phi \cdot A \leq C_2: P = \frac{B_\phi A - C_1}{C_2 - C_1} \quad (2)$$

$$C_2 \leq B_\phi \cdot A: P = 1$$

Whether the tube leaves the shear region or not is determined by a random number generator, according to the above defined probabilities. The relevant poloidal field component, B_r , is regenerated by an unspecified dynamo process yielding a current parallel (Northern Hemisphere) or antiparallel (Southern Hemisphere) to the toroidal field B_ϕ . The radial component of the field produced by this current for a flux tube situated at $x = x_0$ is assumed to have the triangular profile $R(x - x_0) = R(\bar{x})$, for simplicity.

$$R(\bar{x}) = \begin{cases} \frac{\bar{x}}{x_m} & \text{for } |\bar{x}| \leq x_m \\ \frac{\bar{x} - \text{sgn } \bar{x} \cdot D}{x_m - D} & \text{for } x_m \leq |\bar{x}| \leq D \\ 0 & \text{for } D \leq |\bar{x}| \end{cases} \quad (3)$$

The parameters x_m and D define the location of the maximum and the total extension of the poloidal field produced by one flux tube, respectively. Therefore, D is a measure for the efficiency of (turbulent) diffusion in dispersing the weak poloidal fields spatially. The regeneration term for the poloidal field coming from one flux tube at $x = x_0$ is hence:

$$r(x, x_0) = \alpha(x_0) B_\phi(x_0, t) R(x - x_0) \quad (4)$$

where $\alpha(x)$ measures the strength of the regeneration process. As the role of rotation and Coriolis forces seem essential for any relevant mechanism we take $\alpha = \alpha_0 \cos x$. Now consider a distribution of N flux tubes at $x = x_1, \dots, x_N$. The poloidal field at the location x_i of one flux tube which is necessary for the amplification of $B_\phi(x_i)$ throughout equation (1) is given by

$$\frac{\partial B_r(x_i)}{\partial t} = -\gamma B_r(x_i) + \sum_{j=1}^N r(x_i, x_j) \quad (5)$$

The contributions of all flux tubes to the poloidal field are summed and $-\gamma B_r$ is a simple dissipation term. (A dissipation term for the toroidal field has not been included in equation (1) because turbulence is assumed to be suppressed within the flux tubes and the molecular diffusivity is neglected. This is not possible for the much weaker poloidal field.)

Equations (1) and (5) have been integrated forward in time, paying attention to flux eruption determined by equation (2) through random numbers. In spite of the considerable number of free parameters, the results sensitively depend only on a few of these. The quotient α/γ determines whether the dynamo is excited or not: a critical value has to be exceeded. The other sensitive parameter is D , the half width of the regenerated poloidal field due to one flux tube. To get a butterfly diagram comparable with the observations $D \geq 0.1 \pi$, otherwise the butterfly wings overlap severely or the large-scale structure changes totally to irregular fluctuations. Therefore, even a flux tube dynamo depends on strong turbulent diffusion allowing for effective superposition of the poloidal field contributions of the individual flux tubes.

The results for a model with $D = 0.125 \pi$, $x_m = 0.025 \pi$ are shown in Figs 1 and 2. Figure 1 shows a 'synthetic' butterfly diagram in which the latitude of erupted flux tubes is drawn against eruption time. Numbers indicate positive field strength (in arbitrary units), letters negative field strength ($A \triangleq -1$, $B \triangleq -2 \dots$). Although only the time of the first appearance of a flux tube is shown, activity caused by it can last for months. This must be remembered when Fig. 1 is compared with observed butterfly diagrams showing broad wings. We can also define an activity index by monitoring the number and field strength of erupting flux tubes per time interval. This index is given in Fig. 2 together with the age of erupted flux tubes as a function of time. Looking first on the activity index, we observe a fast rise of the cycle, followed by a slower descent towards the minimum in accordance with the typical behaviour of the sunspot cycle (the secondary maximum is possibly accidental).

The cyclic variation of flux tube age seems to be very significant. The youngest tubes appear at activity maximum, flux tube age increases gradually during the decline of activity and reaches a maximum at activity minimum, followed by a sharp falling-off during the rise to the activity maximum. This variation is almost identical to the behaviour of sunspot brightness in time^{5,6} and supports our hypothesis. Moreover, the anticyclic variation of XBP and EAR can be interpreted as an effect of age, too: older and therefore more severely 'shredded' tubes appear about activity minimum. It is quite plausible that the oldest of them are disrupted totally and only appear as XBP and EAR in high latitudes as the first messengers of the new cycle⁴. This would also decrease the latitude at which the first spots of a new cycle appear and thus remove the excess extension of the butterfly wings in Fig. 1 (XBP and EAR are not included in the classical activity measures, sunspot numbers and butterfly diagram). The problem of activity appearing in high latitudes was encountered by most dynamo models and was usually dealt with by ad hoc arguments.

Results from this crude and simplified model are thus very encouraging: the variations of sunspot brightness and of the appearance of XBP and EAR can be interpreted as an effect of age of the basic flux tubes. In considering the 'solar clock' proposed by Dicke¹⁴ and the Maunder minimum, the present calculations, Leighton's and many other dynamo models show that non-linear dynamo action has a self-stabilization of phase, even if random fluctuations are allowed for.

Phase fluctuations in certain activity measures as sunspot number or the D/H indicator can easily be interpreted as fluctuations in the ratio of magnetic flux appearing in EAR to the flux appearing in big active regions with spots and flares, that is fluctuations of the amplitude of dynamo action (poloidal field), not phase. As the dynamo operates deep down in the convection zone with constant phase, then phase fluctuations of surface activity do not lead to a random walk of phase: the dynamo itself is the solar chronometer. The low sunspot numbers during the Maunder minimum may not be interpreted by a total stop of the 'machine', but can be understood as a period of weaker poloidal field causing the production of older flux tubes which have been disrupted by convection and only appeared as EAR, not sunspots. The dynamo has operated all the time and no phase problems arise at the end of this period.

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X-ray diffraction from adsorbed iodine on graphite

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Our knowledge of surface structure at the solid-vacuum interface has been acquired largely through high-vacuum analytical techniques. Unfortunately these may not be applied *in situ* to solid-liquid or solid-high-pressure gas interfaces which are of greater practical importance. *Ex situ* investigations have been made but the transfers involved probably result in surface restructuring. It is therefore apparent that *in situ* techniques are required. Raman spectroscopy¹ is applicable but lacks versatility, whilst neutron diffraction² and SEXAFS³ are promising but depend on a reactor and synchrotron respectively. X-ray diffraction, on the other hand, is routinely performed and the theory is well developed though it has been assumed to be insensitive to surfaces. We report here the first observation of X-ray diffraction from a two-dimensional adsorbate formed at the solid-liquid interface, I_2 on carbon, using position-sensitive detection to enhance sensitivity.

The adsorption of I_2 from aqueous KI_3 solution, on carbon was chosen as a test system as it has been widely studied and also because it is particularly suited to X-ray investigation in view of the large scattering cross-section of I_2 with respect to carbon. The adsorbent chosen was Papyex; a graphitic material (in the form of 0.15-mm thick sheets) in which approximately half the graphite basal planes lie within a few degrees of parallel to the surface. This material has recently found wide use in neutron

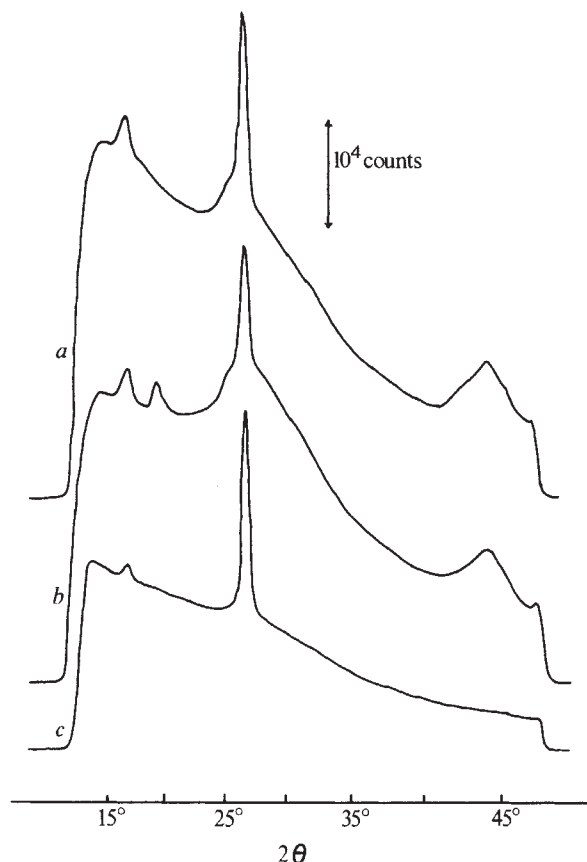


Fig. 1 Diffractograms of Papyex in: *a*, 0.1M KI, in-plane geometry; *b*, 0.02M I₂ in 0.1M KI, in-plane geometry; *c*, 0.02M I₂ in 0.1M KI, out-of-plane geometry. The sample thickness for *a* and *b* was 0.3 mm and for *c* 0.8 mm. The count time was 4,000 s.

diffraction studies of gas-phase adsorption⁴ and has a surface area of $\sim 20 \text{ m}^2 \text{ g}^{-1}$. Whilst higher surface area graphites are available, the preferred orientation of the graphite flakes in Papyex make it particularly useful; one can perform diffraction experiments either in the in-plane geometry (X-ray beam normal to the surface) probing primarily correlations in the plane of the surface or (by turning the sample through 90°) in the out-of-plane geometry, probing correlations perpendicular to this plane.

In the experiments reported here, Papyex sheets were equilibrated for 48 h with aqueous solutions of I₂ in KI before transfer to a cell fitted with Mylar windows for the entrance and exit of the X-ray beam. A stack of two Papyex sheets was used and the cell was designed to minimize the thickness of solution through which the beam passed.

The scattering from an adsorbate is obviously very small compared with the total observed (the substrate and H₂O molecules contribute the major portion). A high photon count is therefore necessary to obtain adequate signal-to-noise ratios. This precludes the use of conventional counting techniques because of the prohibitive times involved. However, the past few years has seen the introduction of X-ray position-sensitive proportional counters (PSPC) which have proved very useful in biological applications where low exposure times are desirable⁵. A PSPC similar to that described by Borkowski and Kopp⁶ was used in this study. Briefly, the anode is a high-resistance wire, parallel to the entrance window, which behaves as a distributed delay line. The position on the wire of the charge pulse caused by the X-ray photon is determined by the risetime of the pulse at each end of the wire and the output of the signal-processing electronics is a pulse, the height of which is related to the position of the X-ray event. These pulses are then sorted by a multichannel analyser which displays the intensity-position spectrum of the diffracted X-rays. The detector used had a

20-cm active length, a window height of 1 cm and a depth of 1 cm. A copper anode X-ray tube operating at 40 kV, 20 mA was the source.

Typical diffractograms are shown in Fig. 1 for Papyex in I₂-free KI solutions and with a monolayer coverage of I₂, both for in-plane geometry, and for monolayer coverage in the out-of-plane geometry. The peak at $2\theta = 26.6^\circ$ on all the diffractograms is the (002) diffraction of graphite, whilst the broad peak at $2\theta = 44.2^\circ$ is that for the (100) and (101) planes. The peak at $2\theta = 16.3^\circ$ is due to the Mylar windows as is the shoulder on the low-angle side of the (002) diffraction. The peak in Fig. 1*b* at $2\theta = 19.6^\circ$ is assigned to the (10) diffraction of a two-dimensional layer of hexagonally close-packed adsorbed I₂ molecules. Figure 2*a-d* shows this region of the diffractogram in more detail for increasing values of I₂ coverage (as determined by titration techniques which indicated a Langmuir-type isotherm with a maximum coverage of $3.9 \times 10^{-2} \text{ g I}_2 \text{ per gC}$). Whilst the position of the (10) diffraction is independent of coverage, the intensity is proportional to it. This indicates that the forces between the adsorbed I₂ molecules are attractive and that the adsorbate is present as islands. From the half width of the (10) peak the size of these islands may be estimated⁷; for the coverage range 0.1–1.0 this was found to be constant at $110 \pm 20 \text{ \AA}$; a value identical to the crystallite size for adsorbed Kr on Papyex as determined by neutron diffraction⁴. This dimension has been attributed to the size of the graphite flakes. It is, therefore, better to regard the iodine adsorption process as a higher-order phase transition. The position of the (10) peak, in the Warren formalism⁷ for diffraction from a two-dimensional structure corresponds to a layer spacing of $4.6 \text{ \AA}$, which in the hexagonally close-packed structure gives a nearest-neighbour distance of $5.3 \text{ \AA}$, which is slightly less than the value at the

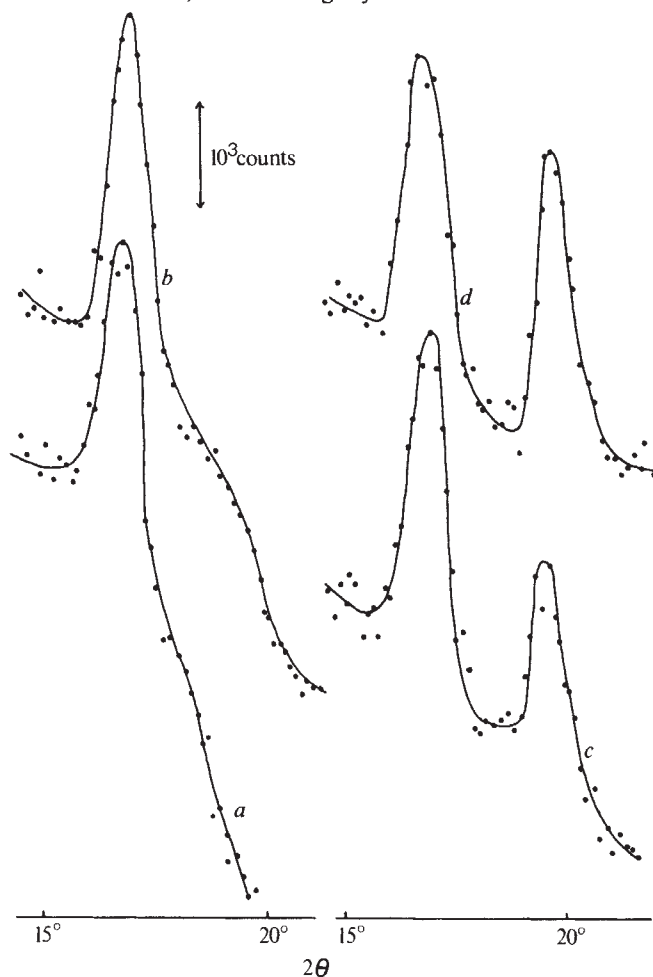


Fig. 2 Details of diffractograms of Papyex, equilibrated with I₂ solutions in KI, between $2\theta = 15^\circ$ and 20° . The coverages were: *a*, 0; *b*, 0.05; *c*, 0.7; *d*, 1.0.

minimum of the Lennard-Jones potential, 5.6 Å. The calculated nearest-neighbour distance, in conjunction with the value of the maximum coverage, gives the surface area of Papyex as $22 \text{ m}^2 \text{ g}^{-1}$, in close agreement with values obtained by other methods.

While these results were obtained for a favourable system, they indicate that X-ray diffraction can be successfully applied to adsorption studies in condensed media. It is inherent in these studies that there will always be a large background signal arising from scattering by the solution, therefore, accurate background subtraction techniques are required. These subtractions are difficult because of the large X-ray absorption cross-sections of potential adsorbates. This is overcome in diffraction studies of gas phase adsorption by recording the background at a temperature where the adsorbate is present as a 'liquid' and not an ordered array, but such a technique is not possible with condensed phases. In electrochemistry, however, it is possible to modulate the electrode potential and therefore accurate background subtraction should be possible. Experiments of this type are now in progress.

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TiO₂ on and around a deactivated hydrodesulphurization catalyst

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Deactivation of catalysts used to promote the liquefaction and desulphurization of coal is usually attributed to structural changes in the catalyst and the buildup of carbonaceous materials, minerals, iron and titanium. We discuss here the nature of the titanium which, though it is one of the major metals to deposit on and around catalysts, has never been characterized. The titanium deposited around a used catalyst removed from a fixed-bed reactor is very similar to one of the TiO₂ polymorphs (anatase) except that the *c* axis is 0.054 Å shorter. Two possible explanations for the origin of the anatase-like material are (1) that the titanium was originally organically bound in the coal, but it became oxidized during coal liquefaction, or (2) that small crystallites of the oxide existed in the coal and were simply concerned on and around the catalyst during liquefaction.

The need for clean fuel sources has led to the development of new processes for the liquefaction of coal. Some, such as the solvent refined coal (SRC) processes, do not use a catalyst, apart from the effects produced by the mineral matter in the feed coal. Others, such as the H-coal process, use an added catalyst, typically Mo promoted with Co or Ni supported on high surface area aluminas. Present usage requires ~1 lb of catalyst with 10% Mo as metal to hydrodesulphurize 1 ton of coal and produce three barrels of liquid fuels; thus it would take ~10 × 10⁶ lb of Mo per year to produce one million barrels of liquid per day. (A production of approximately this amount would be required for a catalytic process to influence significantly the generation of liquid fuels in the US.) Because US production of Mo was estimated at 65 × 10⁶ lb in 1978 and world production was 194 × 10⁶ lb the need for lifetime maximization for Mo-based catalysts is obvious¹.

Catalyst deactivation has been extensively studied for both petroleum and coal hydrodesulphurization catalysts²⁻⁴, but the nature of the titanium in the latter was not determined.

Much work at the Pittsburgh Energy Technology Center (PETC) has been devoted to a study of deactivated catalysts (supported Co-Mo on alumina) removed from the SYNTHOIL (fixed-bed) reactor. After 300 h of operation at 450 °C, 4,000 p.s.i., and exposure to 1.3 tons of Homestead, Kentucky coal, the 11 lb of Mo catalyst had become deactivated, as shown by the increase in sulphur content of the liquid product from 0.25 to 0.75%, the latter value equivalent to that found in non-catalytic operations⁵. The studies at PETC have shown that part of the catalyst deactivation may be due to the growth of the MoS₂ crystallites from single layers into three-dimensional stacks containing nine layers⁶. The work reported here is limited to the nature of the titanium deposits found on and around the spent catalyst.

The titanium found in the catalyst is concentrated in the outer 150 μm of the 1/8-inch catalyst pellets and is difficult to separate from the rest of the catalyst. However, surrounding the pellets in the reactor is a greyish deposit similar in composition to the spent catalyst except for lower Al, Mo, and Co contents. (The unused catalyst contains 3% CoO, 15% MoO₃ and 5% SiO₂ on eta Al₂O₃.) An X-ray diffraction pattern of this deposit, which contains ~20% Ti, is shown in Fig. 1 *b*. The anatase form of TiO₂, with lines slightly shifted, is the major crystalline compound in the sample. Calcite, gypsum and quartz which occur in the original coal and pyrrhotite which forms during liquefaction are also present. The strongest line of anatase at 3.52 Å can easily be seen on diffraction patterns of the whole unground pellets, but long counting times are needed to reveal the second-strongest line at 1.89 Å.

The composition of the reactor deposit (shown in Fig. 1) is listed in Table 1. For comparison the analysis of used catalyst from the same portion of the reactor, the outlet side, is also shown.

After treatment with HCl and HF, all but ~15% of the reactor deposit dissolves. (Although Vorres and Donohue⁷ showed in 1955 that TiO₂ reacts with HF to form TiOF₂, this information does not yet seem widely disseminated.) The acid-insoluble residue contains a coke-like material, MoS₂, and an unidentified magnetite-type spinel (see Fig. 1*a*). From X-ray diffraction linewidth measurements, corrected for instrumental broadening, the crystallite size of the anatase-like material in the reactor deposit was estimated⁸ to be in the range 190-260 Å. The positions of the peaks, except the (200), are shifted to

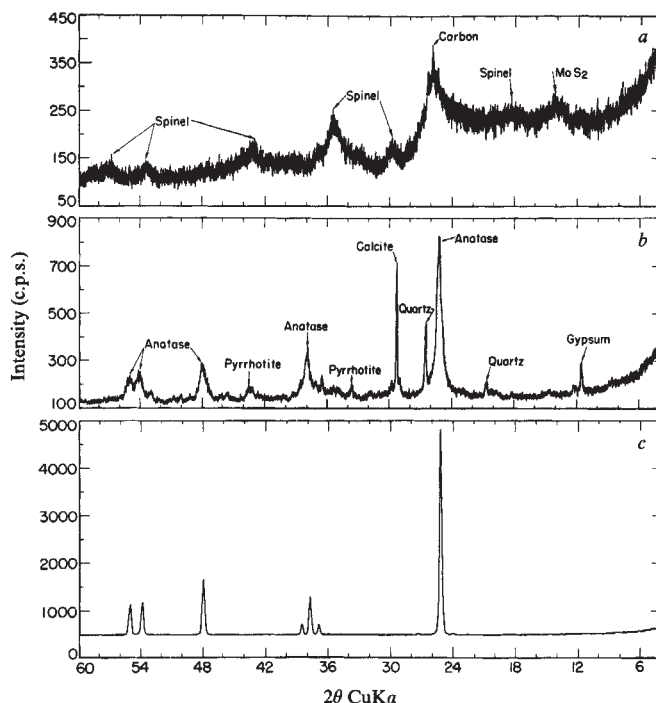


Fig. 1 Diffraction patterns of: *a*, Reactor deposit that has been treated with HCl and HF; *b*, reactor deposit; *c*, pure anatase.

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Table 1 Selected metals, *C* and *H* content of reactor deposit and used catalyst pellets from outlet section of SYNTHOIL reactor

Element	Reactor deposit %	Used catalyst pellets %
Ti	24.5	2.9
Fe	7.82	0.5
Al	7.81	30.1
Ca	1.74	0.3
Mo	1.92	7.8
Co	0.55	1.2
C	15.3	9.8
H	1.3	0.9

slightly higher angles, and from measurement of six peaks, a *c* axis of 9.460 Å with a standard deviation of 0.006 Å has been calculated using Appleman's⁹ least-squares program. This indicates a decrease of 0.054 Å from that measured by Swanson *et al.*¹⁰ for pure anatase. No change in the *a* axis was detected. The reason for the slightly shorter *c* axis of the anatase-like material in the reactor deposit is not known. This material also contains ~1% boron, but when treated with HCl, the boron is found in the soluble fraction, whereas the titanium-rich material is insoluble. Scanning transmission electron microscopy (STEM) studies showed no element other than Ti in the anatase-like crystallites from the reactor deposit. Attempts have been made to study by STEM the TiO₂ on and in the pellets but the preparation of TiO₂ crystallites thin enough to produce electron diffraction patterns has not been achieved.

A sample of the raw coal (Homestead, Kentucky) was treated to concentrate any TiO₂ minerals present. The coal was first ashed at low temperature and then fractionated using bromoform. HCl and HNO₃ were then used to remove calcite and pyrite from the residue. No titanium minerals were detected in the residue even though the coal contains ~0.1% titanium. Quantitative estimates of the inorganic titanium in the Homestead coal have not been made, but Finkelman¹¹, using a novel procedure based on SEM and energy-dispersive X-ray fluorescence, has attempted to do this for a Waynesburg coal. He found that approximately half the titanium is in minerals >2 µm in diameter, and the remainder of the titanium is either organically bound or in smaller crystals. The presence of 200-Å anatase crystallites in the Homestead coal is possible because Lin *et al.*¹² have found mineral crystallites 40–1,000 Å in an Illinois no. 6 coal, although they did not specifically find anatase.

Recent work here at PETC has shown that titanium bound as the cresylate, nonylate, or tetraphenyl porphine, when mixed with catalyst, penetrated several hundred micrometres into the pellets. After treatment in an autoclave at 450° and 2,000 p.s.i. hydrogen for 30 min, the titanium in between the pellets had been converted to 600–800-Å anatase crystallites with normal *a* and *c* axes, and the structure of the titanium inside the pellets is under investigation. This work shows that organically bound titanium will form anatase in liquefaction conditions. However, until more conclusive studies rule out the presence of small crystallites of TiO₂ in the original coal, the inorganic origin remains a possibility. Scanning transmission electron microscopy studies and additional differential solubility experiments to concentrate any inorganic TiO₂ present in the raw Homestead coal are in progress. Hopefully, this information will permit resolution of the origin of titanium deposits on at least one coal hydrodesulphurization catalyst.

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Olivine glass and spinel formed in a laser heated, diamond-anvil high pressure cell

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Laser-heated, diamond anvil cells have produced a major breakthrough in the understanding of the physics of the Earth's interior, by making it possible to synthesize mineral phases that are stable at very high pressure. The synthesized phases are usually identified by X-ray powder diffraction techniques. We report here the results of the first local examination by transmission electron microscopy of a sample prepared by heating fayalite in a diamond-anvil cell. We have identified three phases produced, in the pressure gradient from the edge to the centre of the cell: olivine glass (to our knowledge, the first case to be reported); phases corresponding to a transition zone between olivine and spinel; and spinel. The phases are similar to those found in shocked chondritic meteorites.

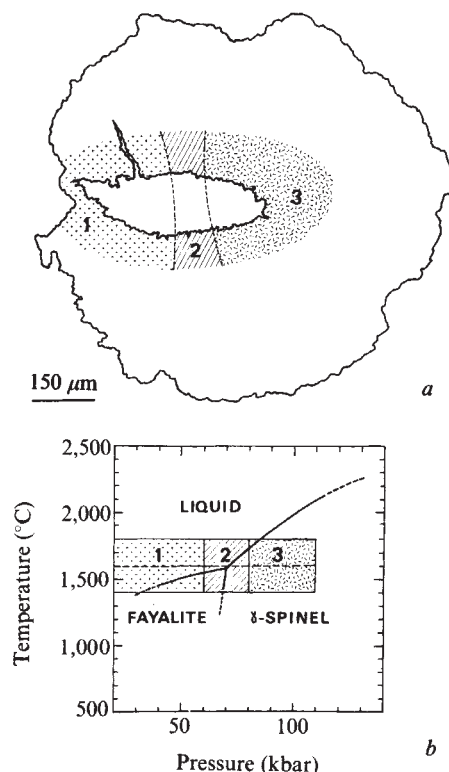


Fig. 1 *a*, Scheme of a sample recovered from the diamond-anvil cell and ion-beam thinned. The edges of the hole are transparent to the electrons. *b*, Location of the different zones of the sample in the phase diagram after Ohtani. Zone 1, glass quenched from the melt at lower pressure near the rim. Zone 2, transition zone. Zone 3, γ-spinel at higher pressures near the centre.

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Fayalite Fe_2SiO_4 is the iron end member of the ferro-magnesian olivine series, which constitute most of the Earth's upper mantle, and whose transition to spinel structure under high pressure has important geodynamic consequences. Pure, single crystalline, synthetic fayalite prepared by zone-melting³, was ground in an agate mortar; the powder, consisting mostly of grains $\sim 3 \mu\text{m}$ in size with a few larger ones ($\approx 30 \mu\text{m}$), was loaded between the diamonds, without a gasket, as a disk 0.8 mm in diameter. Pressure was applied for 2 days before heating. The maximum pressure, measured by the shift of ruby fluorescence lines, was 109 ± 10 kbar in the centre of the specimen and was found to be the same when measured before and after heating. The distribution of pressure across the cell was not measured but the absence of a gasket meant that the pressure at the edge would be close to atmospheric pressure. The beam of a 60-W YAG laser was focused on the sample and scanned across it. The powder density was of the order of 1 MW cm^{-2} and the luminance temperature was estimated to be $1,450^\circ\text{C} \pm 50^\circ\text{C}$ by optical pyrometry. The sample was then recovered and ion-beam thinned until a hole appeared. The elongate hole extended from the centre of the sample to the edge of the transformed zone (Fig. 1). Its edges were thin enough to be examined by transmission electron microscopy in a 100-keV Philips EM300 microscope. From the rim to the centre three structurally different zones were distinguished and could be interpreted in the light of Ohtani's recent phase diagram for melting of Fe_2SiO_4 under pressure⁴ (Fig. 2). These results were reproduced in two experiments with nominally the same conditions. (In one experiment the recovered sample broke but fragments could be thinned and examined, they yielded the same results).

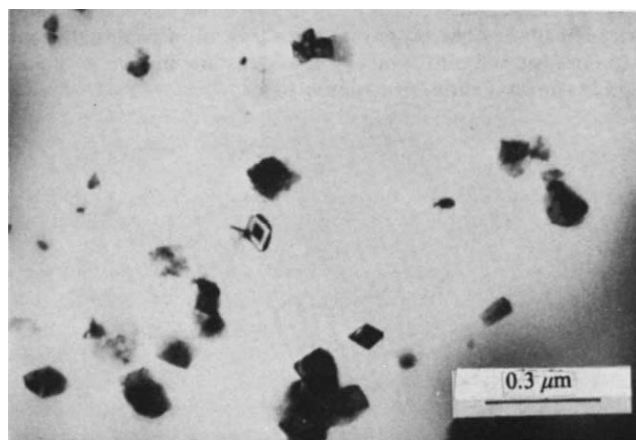


Fig. 2 Fayalite glass with small fayalite crystals in zone 1.

Zone 1, close to the rim, consists mostly of a material which was totally nondiffracting, melted easily when heated in the beam by removing the condenser apertures of the microscope (this prevented X-ray microanalysis), and contained a few idiomorphic micro crystals of a size much too small ($< 1,000 \text{ \AA}$) to allow characterization by electron diffraction. These three features together with the fact that the pressure at the rim is low enough for fayalite to melt under the impact of the laser beam lead us to believe that we have observed olivine glass quenched from the melt. We believe that this is the first report of an olivine glass quenched from the melt, the only glass previously reported having been produced in the solid state during shock experiments⁵. The highly conducting diamonds can evacuate heat from the very thin layer of melt, fast enough to obtain very high quenching rates comparable with rates ($\approx 10^6^\circ\text{C s}^{-1}$) obtained in the preparation of metallic glasses⁶. We can get an idea of the quenching rate by a rough calculation assuming that a quantity of heat $Q = 2\lambda\rho cT_0$ per unit area is released instantaneously at

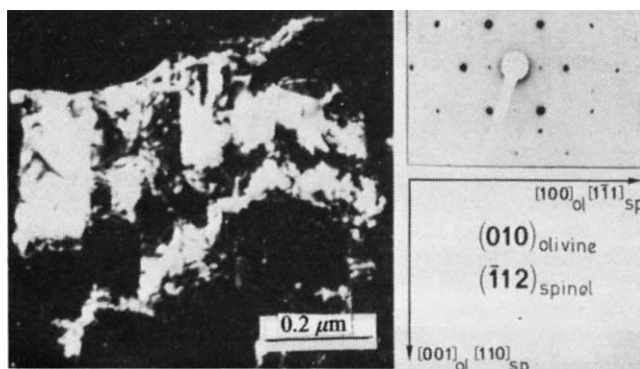


Fig. 3 γ -spinel and olivine intergrowths in the transition zone 2. The plane of the foil can be indexed as (010) in olivine or (112) in spinel, the boundaries between the areas in and out of contrast are (100) planes in olivine or (111) planes in spinel.

time $t = 0$ in a thin layer of thickness 2λ at $x = 0$ (the sample) and evacuated on both sides by conduction in semi-infinite diamond anvils along the x axis. If we assume that ρc (density $\times$ heat capacity) is not very different in the thin sample and in the diamonds; the temperature T at time t and $x = 0$ (the temperature of the sample) is given⁷ by: $T = T_0\lambda/(\pi\kappa t)^{1/2}$ where $\lambda \approx 10^{-3} \text{ cm}$ and κ is the thermal diffusivity of diamond, $\kappa \approx 1 \text{ cm}^2 \text{ s}^{-1}$, T_0 is the temperature of the liquid $T_0 \approx 1,500^\circ\text{C}$ (the temperature of the diamonds at $t = 0$ is taken as 0°C). We see that for $t = 10^{-6} \text{ s}$, T falls to $\sim 850^\circ\text{C}$, well below the freezing point: the quenching rate is probably higher than $10^6^\circ\text{C s}^{-1}$.

Zone 2, inwards of zone 1, probably corresponds to the vicinity of the triple point liquid-fayalite- γ -spinel, in the solid state region. It seems to be a transition zone between the olivine and spinel structure, where the electron diffraction patterns can be indexed both as (010) plane in olivine and (112) plane in spinel (Fig. 4). If various spots are used to form the image, different tabular regions of the grains are seen in contrast, the boundaries between these regions lie on (100) plane in olivine or (111) plane in spinel. This structure could be interpreted as corresponding to conditions close to the olivine-spinel boundary in the phase diagram, and is compatible with a model recently proposed⁸, of a martensitic olivine-spinel transition effected by the motion of dissociated [001] dislocations on the (100) close packed planes of olivine, with accompanying synchroshear of the cations.

Zone 3, close to the central region, consists only of γ -spinel. Further from the centre the grains are quite small, $1 \mu\text{m}$ in diameter, with a polygonal shape; they contain planar defects (stacking faults or antiphase boundaries) (Fig. 4) on (110) planes

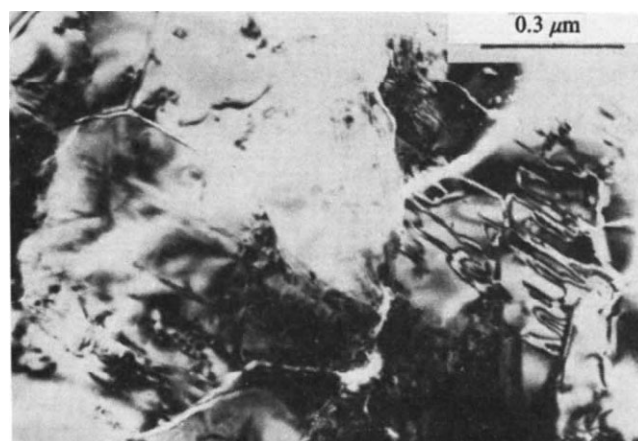


Fig. 4 γ -spinel with antiphase boundaries in zone 3.

with displacement vector $1/4[110]$, identical to the defects previously identified in the ringwoodite of the meteorite Tenham⁹⁻¹¹. Closer to the centre, the grains become larger (several micrometres), planar defects are no longer visible but numerous straight dislocations aligned along $\langle 110 \rangle$ directions are present.

In conclusion, transmission electron microscopy has shown that processes taking place in fayalite inside a laser-heated diamond-anvil cell are rather complex and range from melting and olivine glass formation during rapid quench to solid state transition to spinel. Note that the observed phases: glass with small idiomorphic crystals and mosaic spinel polycrystal with antiphase boundaries are very similar to those found in shocked hypersthene chondrites.

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Phytoplankton patchiness indicates the fluctuation spectrum of mesoscale oceanic structure

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Satellite imagery and large-scale oceanic experiments have replaced traditional concepts of broad sluggish currents moving throughout the ocean by the concept of a continuous distribution of more energetic eddies, the dominant eddies having space scales^{1,2} of the order of 100 km. Near the ocean boundaries in such areas as the Gulf Stream and the California Current, these eddies exhibit strong thermal contrast, of the order of 2-6 °C, and their structure and distribution can be mapped using satellite thermal IR imagery^{3,4}. Over most of the ocean, however, the thermal contrasts are much lower, of the order of 0.1-0.5 °C, and tend to be masked from satellite observation by small-scale atmospheric variations. To map the structure associated with mesoscale horizontal water motions in these areas, another indicator must be used for observation from space. We present here the first results of spectral analysis of satellite imagery of what we believe is phytoplankton patchiness controlled by mesoscale (10-100 km) water motions.

The surface layer of the ocean is inhabited by organisms ranging in scale from plankton (~1 µm) to whales (~10 m). Whereas whales often migrate over hemispheric distances, phytoplankton, the microscopic plants in the ocean, have the least mobility, limited to vertical migration over a few metres per day⁵ accomplished by buoyancy changes. The phytoplankton may, therefore, be considered as a passive scalar being advected about by the variable ocean currents. Phytoplankton do, however, grow and reproduce; a healthy population can double its biomass in a time period of the order of 1 day⁶. Several

models have been developed to describe growing phytoplankton patches being redistributed and diffused horizontally by a partially turbulent ocean⁷⁻⁹. These models have tried to describe the observed spatial variations of biomass in terms of wavenumber spectra. It has recently been concluded^{7,8,10} that shipborne sampling cannot achieve sufficient spatial coverage in times short enough to be considered synoptic and that airborne or satellite sampling is required.

The biomass of phytoplankton is usually quantified by determining the amount of chlorophyll *a* pigment present. Remote measurement of chlorophyll concentration uses the colour changes caused by the combination of absorption or fluorescence of chlorophyll pigments and backscattering by associated suspended matter¹¹⁻¹⁵. Variations in phytoplankton concentration cause measurable changes in the intensity and spectral distribution of visible light backscattered from the ocean, especially in the blue and green bands where water transmission is a maximum. Normal, low plankton concentrations cause colour changes which can be detected from space only by using specialized processing on selected wavelength bands to remove atmospheric effects. The Coastal Zone Colour Scanner on Nimbus 7 is now available as a sensor designed for this purpose. Areas of high chlorophyll concentration, however, can give large changes in water reflectivity detectable by less sophisticated systems.

Figure 1 shows an area of the ocean 180 × 250 km in size, south of Iceland, as imaged by the LANDSAT multispectral scanner on 19 June 1976. The image shown is a mosaic of spectral band 4 from frames 2514-12015 and 2514-12021. Band 4 is the shortest wavelength band on the instrument, covering 500-600 nm, corresponding roughly to green light. The south-west coast of Iceland is visible at the top of the image, and the 400-m depth contour crosses the middle of the scene. The land is partly cloud covered and some bands of cloud are visible at the lower right. The island of Westmannaeyjar can be seen just off the coast with the volcanic island, Surtsey, to its south-west. The swirls and eddies in the remaining area of the water show a structure characteristic of mesoscale turbulence as observed in satellite thermal imagery. This structure is much less visible in the red band, while the longest wavelength band 7 (800-1,100 nm) shows only the clouds above featureless dark water. Because this band-spectral signature is typical of suspended matter in the ocean, we interpret the eddy patterns in Fig. 1 as representing the distribution of phytoplankton biomass or of detritus derived from phytoplankton.

A phytoplankton survey tow was made in the area as part of a programme of the Institute for Marine Environmental Research, UK, on the day this image was taken. The ship travelled westwards parallel to the coast ~20-km offshore passing between Westmannaeyjar and Surtsey. The concentration of phytoplankton observed was well below average for the time of year confirming, at least, that the darker area near the top of the image corresponds to a low concentration of phytoplankton. Measurements made along the same line six weeks earlier, however, showed the strongest phytoplankton bloom observed in this area during May for five years. Repeated measurements made on a section to the west of Westmannaeyjar by staff of the Marine Research Institute of Reykjavik¹⁶ showed that the main peak of growth in this area also occurred about a month earlier. Their data for 1976-78 show that the blooms tend to occur earlier in the shallower water on the continental shelf than in the deeper water offshore where the bloom in Fig. 1 is observed. These data also showed that an exceptionally high productivity of phytoplankton occurred in the spring of 1976. The available data, then, confirm strong productivity near this place and time and do not invalidate our interpretation of Fig. 1 as showing the effects of a phytoplankton bloom. The area of the bloom covers at least 180 × 180 km, the area of a single frame of LANDSAT imagery. The increase in reflectance caused by the bloom is high, but not unreasonable for plankton of the appropriate species¹¹ or in the appropriate stage of development¹⁷. No other LANDSAT scenes were collected over the area near this time

except for an image overlapping the area of Fig. 1 but farther east, largely cloud covered, recorded on 30 May. This image shows no discernible bloom. Scarcity of satellite data is largely due to there having been no satellite receiving station capable of receiving directly image data of Iceland.

The characteristic spatial scales of the eddies and ocean turbulence structure can best be determined from a two-dimensional spectral analysis¹⁸ of the image in which we assume that the increase in pixel value over the level for clear water is proportional to phytoplankton concentration. The resulting power spectrum can then be averaged over all directions to get a spectrum as a function of a nondirectional wave number¹⁹.

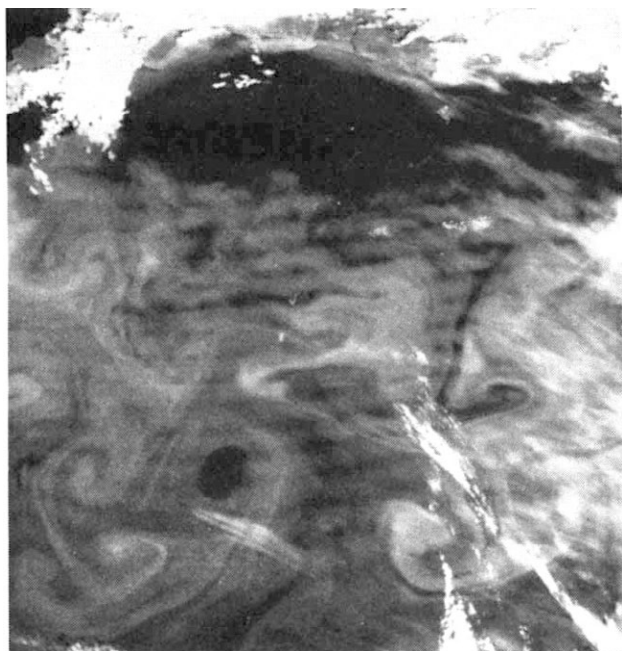


Fig. 1 Enhanced image formed from two LANDSAT Multi-spectral Scanner band 4 frames showing increased reflectance in the Atlantic Ocean south of Iceland on 19 June 1976. The patterns are interpreted as due to a plankton bloom in water advected by ocean mesoscale turbulence.

Before beginning this analysis, the effects of cloud were reduced by subtracting the band 7 image, suitably scaled, from the band 4 image. As water looks very dark in band 7 (near IR), the subtraction is equivalent to removing the light backscattered by aerosol particles in thin clouds and haze above the water. This scaling allows for the different instrument gain and solar irradiance between bands 4 and 7 and also, in effect, selects different values of mean Angstrom coefficient for the spectral dependence of aerosol scattering in the scene²⁰. The scaling constant was adjusted to reduce the visibility of cloud features in the band 4 image. In ~10% of the area of Fig. 1 analysed, thin cloud contributed an additional 15–30% to the radiance enhancement in band 4 that is due to light scatterers in the water. The subtraction reduced this cloud contribution to ~5%. Less correction is possible in areas with thicker cloud, but these were largely avoided in the analysis.

Picture elements in the raw image are spaced on a grid with element spacing 57×79 m. The pixel values were averaged over groups of 8×6 pixels to reduce instrument noise and to give a roughly square grid of about 474-m spacing for further processing. Three overlapping areas of 256×256 averaged pixels each about 120 km square were then selected for Fourier transforming. The mean pixel value for each was subtracted from each pixel in the area, and the areas were Hanned (weighted with a cosine taper in both dimensions) to reduce edge effects.

Although the three areas overlapped by ~55%, the Hanning reduced the correlation of the coefficients of the resulting transforms to ~25%, low enough for reasonable statistical independence. The Fourier transforms of the pixel arrays were then converted to power or signal-variance spectra, and circular ring areas in the transform plane were summed and normalized by their areas to give non-directional estimates of the variance as a function of wavelength. Wedge-shaped areas were similarly summed to give estimates of the directional effects in the water or introduced by residual cloud signals. These were found to be <10% except at the shortest wavelengths (<3 km) where a larger value (up to 20%) appears in the direction range appropriate to the cloud streaks in Fig. 1.

The logarithms of the spectral components of signal variance, averaged over all directions, are plotted in Fig. 2 against the logarithm of the inverse wavelength, K . If the spectral components are dependent on K in a power law manner, proportional to K^n , then such a plot should yield a straight line with slope n . The spectral components computed for the three areas are plotted separately and all yield a similar relation for the wavelength range 2–60 km ($K = 0.015$ to 0.5); the relations can be well fitted by straight lines. The regression line slopes are -3.11 , -2.83 and -2.82 for the three areas giving an average value of -2.92 . The average regression line is plotted as the dashed line in Fig. 2. At long wavelengths, the points lie below this line, but some roll off is expected for components having wavelengths comparable to the dimensions of the sample area. The point for a wavelength of 1 km lies above the line indicating reduction in slope at the short wavelength. This value may be increased by components of scene radiance due to residual cloud reflections. Variance due to small streaks of cloud such as can be seen in Fig. 1 would be expected to have more energy at the higher frequencies.

For wavelengths between 2 and 60 km the distribution of signal variance is related to inverse wavelength to the power -2.9 . If the observed pixel values are interpreted as proportional to particulate backscatter from phytoplankton, then this power law also applies to the phytoplankton density and the above result can be compared with other measurements of the power law index in ocean mesoscale turbulence.

Spectra of sea-surface temperatures derived from airborne radiometer measurements have varied between -2 and -3 (refs 18, 19). Shipboard estimates of phytoplankton patchiness have varied widely, but a similar range is most typical^{10,21–23}. Theoretical estimates^{7–9} of the index for turbulence-controlled phytoplankton patchiness tend to be between -2 and -3 . The -3 slope is consistent with the concept of two-dimensional geostrophical turbulence^{24,25}, where the growth rate of the phytoplankton has a negligible effect on the horizontal distribution of biomass. The signal discontinuities resulting from the small (~10) number of discrete pixel values or grey scale levels in the original image would shift the slope towards -2 (ref. 26), but this effect is reduced to a negligible amount by averaging over 48 pixels.

The relationship between phytoplankton concentration and the amount of radiance upwelling from beneath the sea surface is not in general linear. The absorption by chlorophyllous pigment increases along with the increased backscatter from the suspended planktonic matter^{12,13}. In the range 500–600 nm, this leads to saturation in the reflectance values after an initial increase. Over a limited range of chlorophyll concentrations, however, the relationship can be approximated by a linear function so that the above discussion holds. The reflectance values in the areas analysed range over ~3–7% reflectance. The expected linearity of the reflectance-to-chlorophyll relation depends on the properties of the plankton actually present. The relatively high reflectivity of the water implies a high backscatter to absorption ratio, a condition which improves the linearity of the relationship.

We interpret patterns on a LANDSAT band 4 multispectral scanner image as being due to phytoplankton and show that the fluctuation spectrum is consistent with the phytoplankton dis-

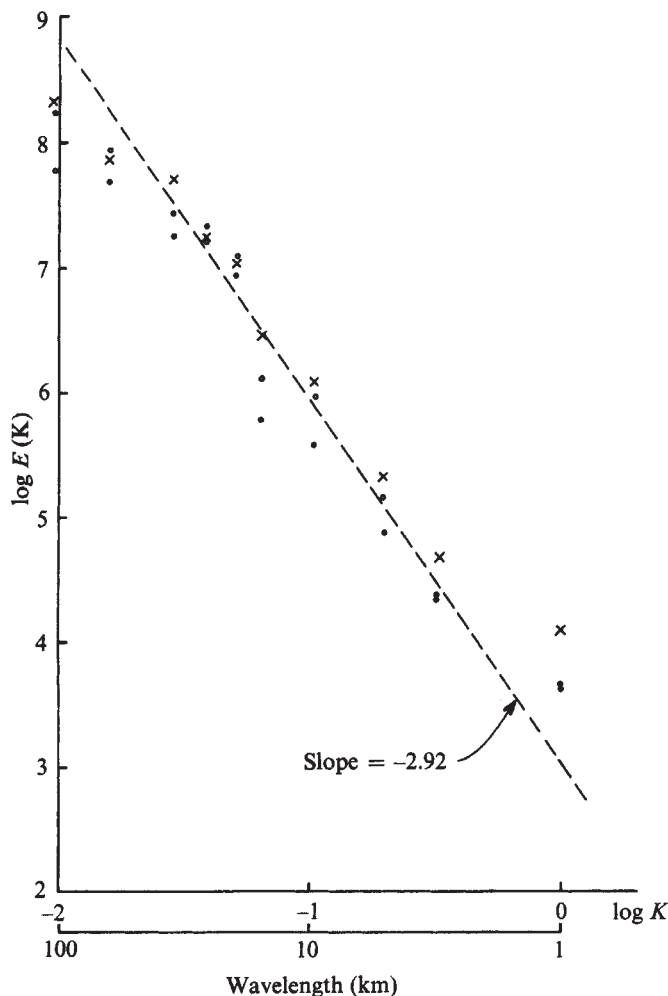


Fig. 2 Power spectra computed from three overlapping areas of Fig. 1 as described in the text. A slope of -3 in a log-log plot is characteristic of turbulent motion, and indicates that growth rate of the plankton itself is having a relatively small effect. The distribution of enhanced water reflectance in Fig. 1 should, therefore, be a useful tracer of the eddy motions.

tribution being controlled by advection in variable ocean currents, while being unaffected by their reproduction rate.

The phytoplankton are, therefore, behaving as a passive scalar and the distribution of enhanced water reflectance in Fig. 1 can be used as a tracer of eddy motion. The spatial distribution of areas showing increased reflectance can certainly be mapped more precisely from a satellite than by any other means available, and this may be why the resulting slope of the power spectrum agrees so well with theory. The problem of relating this reflectance increase to exact measures of chlorophyll concentration remains, but this should soon be possible using the improved data from NASA's Coastal Zone Colour Scanner.

In comparing satellite IR and colour mapping of ocean eddy structure both have their limitations, and the two methods will to some extent be complementary. Both methods are blocked by clouds, and both are affected by atmospheric absorption and scattering. Both measure only surface phenomena, though wind mixing, particularly at high latitudes, will tend to average properties over a surface mixed layer, often of the order of 50 m deep. Thermal measurements are more susceptible to skin effects related to the very small penetration depth (≤ 0.1 mm) of thermal radiation. Both measurements are of ocean properties interesting and important in themselves. Taken together they can show how solar heating, water mixing and stratification, and plankton growth lead to the productivity variations that are of such importance to the world's fisheries.

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The nature of inherited deafness in deafness mice

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Many mouse mutants have an apparent deficiency in their responsiveness to sound¹. Most of these mutants have other abnormalities in addition to their hearing deficit, and the only two which have been subjected to a detailed anatomical and physiological study, *shaker-1* and *Ames waltzer*, also have motor abnormalities^{2,3}. The existence of such motor abnormalities throws some doubt on the usefulness of these two mutants as possible models for hereditary deafness in man, which is most frequently uncomplicated⁴. Deol and Kocher have described the *deafness* mutation in which mice homozygous for the recessive *deafness* gene (*dn/dn*) were unresponsive to sound and had no significant behavioural abnormality⁵. Cochlear hair cells in *deafness* mice develop normally and then degenerate, and the adult animals are completely deaf⁶. We have now studied *deafness* mice in order to determine the nature of their inherited deafness. Our data indicate that stimulus-related cochlear potentials do not develop even though hair cells are present in the young animal. The endocochlear potential is present in the scala media, but behaves abnormally during anoxia.

Deafness mice were bred in our laboratory from stock obtained from M.S. Deol, University College, London. Heterozygotes (*+dn*) were used as controls, and were distinguished from their homozygous (*dn/dn*) littermates by the failure of the latter to exhibit a Preyer reflex in response to a metallic click. Gross VIIIth nerve action potential thresholds for control animals are presented in Fig. 1. No action potentials could be detected in *deafness* mice in any age group at any frequency, even when 5,000 stimulus repetitions were included in the average.

Cochlear microphonics in response to discrete continuous tones between 1 kHz and 60 kHz were recorded from the same electrode and measured with a computer-controlled lock-in amplifier. No cochlear microphonic could be recorded at any age in the mutant mice, although normal microphonics with maximum amplitudes of about 100 μ V were found in control animals. Resting noise levels for these measurements were below 0.1 μ V.

After the electrophysiological experiments, the cochleas were removed and fixed in a buffered paraformaldehyde/glutaraldehyde fixative and embedded in Spurr low-viscosity resin. In surface preparations, most of the cochlear hair cells are present and apparently intact in young (13 to 17 day) *dn/dn* mice, but there is a progressive degeneration so that fewer than 15% of the hair cells remain at 40 days. However, 2- μ m thick sections from young *deafness* mice revealed structural abnormalities in the organ of Corti even at the time when hair cells are still present. In particular, the fluid spaces around the outer hair cells and in the tunnel of Corti do not develop normally. There were no gross abnormalities in the stria vascularis.

Endocochlear potential (the positive d.c. resting potential in the scala media) was examined in mice aged 26–30 days. Potentials were measured and anoxia was induced by sealing the tracheal cannula. The data presented in Fig. 2 show that the resting potentials are identical in control and *deafness* animals, but that *deafness* animals do not show the large negative anoxia potential usually observed in scala media during anoxia⁷.

The present study shows a normal pattern of development in control animals⁸, and suggests that *deafness* mice are profoundly deaf during all stages of cochlear development, including the early stages when most hair cells are intact. There are two possible explanations for the absence of microphonics in *deafness* mice: (1) mechanical stimulation of the hair cells is absent, or (2) the mechanism of generating the microphonic from mechanical stimulation is not functioning. The first possibility seems unlikely because we can detect no external or middle ear abnormalities in the mutants. It is generally assumed that the positive endocochlear potential provides a driving force for potassium ions to cross the apical surfaces of hair cells, and that mechanical stimulation controls the opening of ion channels in the hair cell membrane, thus giving rise to the cochlear microphonic^{9,10}. Since the positive endocochlear potential is present in *deafness*, the most likely explanation for the absence of microphonics is that the ion channels are not opening normally. It is not yet clear whether this failure constitutes the primary action of the *deafness* gene, or whether it follows as a consequence of some other genetically determined abnormality.

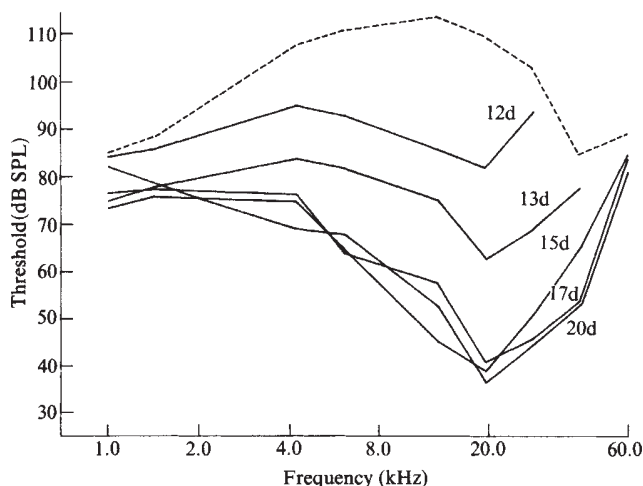


Fig. 1 Mean gross VIIIth nerve action potential thresholds (as sound pressure level in dB SPL) recorded from control mice aged 12, 13, 15, 17, and 20 days after birth. Animals were anaesthetized with urethane (1.5 mg per g intraperitoneally), a tracheal cannula was inserted and the gross VIIIth nerve action potentials were recorded from a silver wire electrode on the round window. Action potentials were amplified, digitized, and averaged on a micro-computer. Responses to tone bursts (40-ms duration, 2-ms rise/fall time) delivered through a closed sound system were recorded, and threshold at a given frequency was taken to be 2.5 dB SPL below the lowest intensity producing a detectable response in an average of 50 stimulus repetitions. Six animals were studied in each age group. The broken line shows the peak output of the sound system at various frequencies.

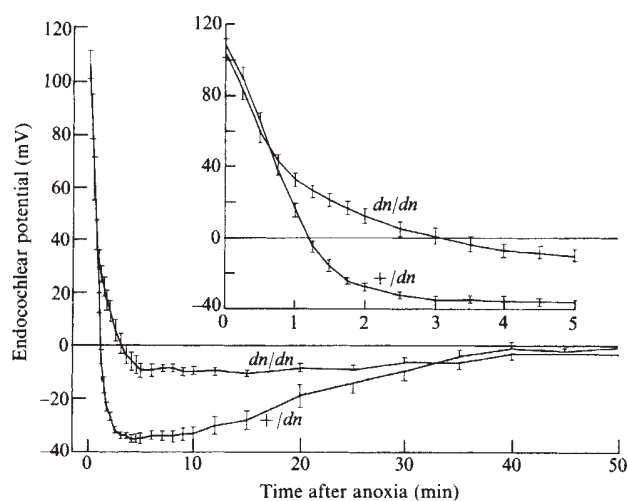


Fig. 2 Animals were prepared as described for Fig. 1 and a glass microelectrode filled with 3 M potassium acetate was inserted into the scala media through a fenestra over the stria vascularis of the basal turn. The potential was recorded as a function of time after the onset of anoxia induced by sealing the tracheal cannula. Each curve shows mean potentials from six animals, and the vertical bars indicate s.e.m. The insert shows the first 5 min of anoxia on an expanded scale. Mean resting potentials were 109 mV (s.e.m. = 2.6 mV) in control animals (+/dn) and 104 mV (s.e.m. = 3.1 mV) in *deafness* animals (*dn/dn*). Mean minimum anoxia potentials were -35 mV (s.e.m. = 1.4 mV) and -9 mV (s.e.m. = 2.7 mV) respectively. Analysis of variance indicated a significant ($F = 48$, d.f. = 1, $P < 0.001$) interaction between genetic constitution and recording condition (resting potential versus anoxia potential).

Recent work on acoustic trauma and ototoxic drug-induced cochlear damage has indicated that an increase in the electrical resistance of the boundaries of the scala media as a result of this damage leads to a decrease in the negativity of the anoxia potential^{11,12}. This is consistent with our suggestion that the ion channels across the apical surfaces of the hair cells are not opening correctly, which would lead to an increase in the electrical resistance, and a reduction in the negativity of the anoxia potential, as observed. However, our endocochlear potential data are difficult to reconcile with the generally accepted mechanism of endocochlear potential generation. It is widely assumed that the resting endocochlear potential is the sum of an active positive electrogenic component and a passive negative diffusion potential, the latter being the only component present during anoxia^{7,13}. The absence of a large negative diffusion potential in *deafness* mice might be expected to lead to the occurrence of a significantly larger positive resting potential compared with the resting potential in control mice. This is clearly not the case in our data. We are currently measuring potassium concentration in scala media of *deafness* mice in an attempt to resolve this problem.

Finally, we suggest that the *deafness* mutant offers a promising animal model of profound hereditary deafness in man. The mutant can be used to study both the primary action of a mutation giving an uncomplicated form of deafness, and the central consequences of the total absence of auditory input during development.

We thank Dr M. S. Deol for providing mice from the *deafness* stock, Mr A. C. Davis for statistical advice and Drs M. P. Haggard and G. K. Yates for critical comments on the manuscript. This research was carried out within the framework of the European Economic Communities' Concerted Action programme for auditory research.

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Genetically determined susceptibility to *Leishmania tropica* infection is expressed by haematopoietic donor cells in mouse radiation chimaeras

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Models of the different disease patterns of cutaneous leishmaniasis can be established by infecting various inbred strains of mice with *Leishmania tropica*. The BALB/c strain is exceptionally susceptible in that the infection is dosage independent and inexorably progressive, terminating in cutaneous and fatal visceral metastasis^{1–3}. This is largely determined by a single autosomal non-H-2-linked gene³, seemingly not identical to the well-characterized *Lsh* gene which determines innate susceptibility to systemic *Leishmania donovani* infection^{4,5}. Although H-2-linked genetic influences are also detectable in the immune stage of both leishmanial infections^{3,6}, they are relatively minor in the case of *L. tropica*. Thus, although progression of disease induced by lower infecting doses is slower in congenic BALB/K (H-2^k) than in BALB/c or BALB/B (H-2^b) mice, the eventual outcome is similar in all three³. This inability to control *L. tropica* infection seems to involve profound impairment of potentially curative cell-mediated immunity by the generation of a specific suppressor T-cell population⁷. With two other pathogens which infect mononuclear phagocytes, *Listeria monocytogenes* and *Mycobacterium lepraemurium*, differences in mouse strain susceptibility have been attributed to extrinsic factors regulating macrophage activity rather than to intrinsic gene expression within the cells themselves^{8,9}. The relative strain susceptibility of mice to myxoviruses (determined by the dominant allele *Mx*) is also a genetic characteristic of their macrophages *in vitro*¹⁰. Nevertheless, radiation chimaeras exhibit the myxovirus resistance profile of the host despite near-total haematopoietic cell replacement, including donor macrophages which can still express their original susceptibility *in vitro*¹¹. In contrast, the experiments described here indicate unequivocally that susceptibility to *L. tropica* in radiation chimaeras compatible at the major histocompatibility (H-2) locus is determined by the descendants of donor haematopoietic cells and not by host environmental factors.

Lethally irradiated mice injected with allogeneic bone marrow cells develop not only haematopoietic and lymphoid cells but also macrophages which are predominantly of donor type^{12,13}. When an H-2 incompatibility is involved, mature T lymphocytes must be excluded from the donor cells to avoid the development of fatal graft-versus-host disease¹⁴. This complication is very rare when untreated marrow is transferred across a non-H-2 incompatibility and the chimaeras survive with extensive replacement of lympho-haematopoietic tissues by donor cells¹⁵. We have adapted this situation to establish reciprocal chimaerism between two H-2-compatible pairs of strains which differ widely in their susceptibility to *L. tropica*³: (1) BALB/B and C57BL/6, respectively highly susceptible and resistant,

both H-2^b; (2) BALB/c and B10.D2, respectively highly and intermediately susceptible, both H-2^d.

BALB/B and BALB/c mice were given 800 rads whole body irradiation, C57BL/6 and B10.D2 900 rads at a rate of 50 rads min⁻¹ from a ¹³⁷Cs source. They were injected the same day with 5 × 10⁶ bone marrow cells either syngeneic or from the H-2-compatible paired strain. Twelve weeks later they were injected over the shaved rump with 2 × 10⁷ viable *L. tropica* promastigotes and the ensuing lesions were observed and measured for 80–100 days as previously described³ (Figs 1, 2). Syngeneically reconstituted chimaeras behaved indistinguishably from intact untreated members of the same strain. With strain pair (1), lesions in BALB/B grew progressively to fatal termination by 80–110 days, whereas in C57BL/6 they remained small and arrested by 20–30 days, with gradual healing. The same two diverging patterns were presented by the allogeneic chimaeras according to the behaviour of the donor: BALB/B → C57BL/6 highly susceptible, C57BL/6 → BALB/B relatively resistant. Complementary findings were obtained with strain pair (2), although here the differences were smaller, reflecting the lesser resistance of B10.D2 than C57BL/6. In this instance, BALB/c → B10.D2 or BALB/c chimaeras were equally susceptible, whereas B10.D2 → BALB/c or B10.D2 showed a similar intermediate profile.

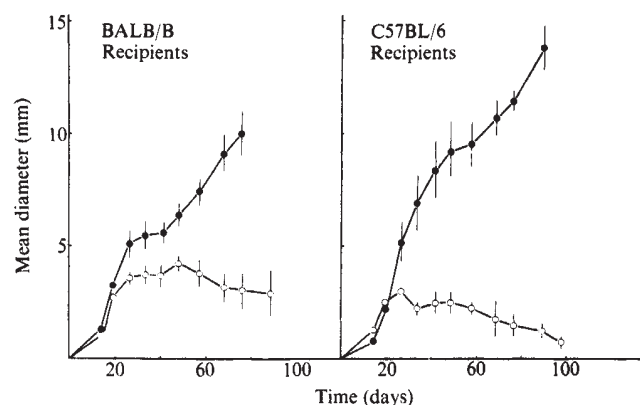


Fig. 1 The course of *L. tropica* infection in H-2^b-compatible radiation chimaeras. BALB/B and C57BL/6 male mice were injected subcutaneously over the rump with 2 × 10⁷ promastigotes 12 weeks after lethal irradiation and reconstitution at age 8–10 weeks with 5 × 10⁶ bone marrow cells from BALB/B (●) or C57BL/6 (○) donors. The mean lesion size ± s.e. is represented (n = 5). (Recipients of BALB/B cells died from disseminated leishmaniasis within 80–110 days.) The susceptibility profiles in both recipient strains are those characteristic of the donor.

These results clearly indicate that the susceptibility to *L. tropica* infection of all four recipient strains studied assumed that of the donor used to establish chimaerism, whether it be more or less resistant. Hence, the exceptional susceptibility of BALB/c and its H-2-congenic derivative lines is expressed intrinsically in some population(s) of haematopoietically derived cells, as this property is transferable by them to a resistant strain. The converse effect implies equally that BALB/c susceptibility is not an environmental influence, in striking contrast with *L. monocytogenes* infection, where reciprocal B10.A and A strain chimaeras have consistently retained resistance characteristic of the host⁸. Similar extrinsic regulation of macrophage activity underlying strain differences in susceptibility to *M. lepraemurium* infection is implicit in recent *in vitro* studies⁹, but this has not been evaluated in chimaeric animals. The fact that radiation chimaeras between strains resistant or susceptible to myxoviruses retain the

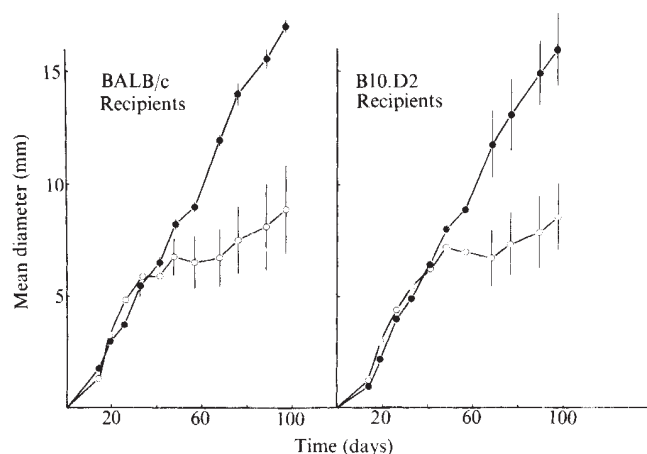


Fig. 2 The course of *L. tropica* infection in H-2^d-compatible radiation chimaeras. BALB/c and B10.D2 mice were irradiated and reconstituted with BALB/c (●) and B10.D2 (○) bone marrow cells, infected and their lesions followed as in Fig. 1 ($n=7$). (Recipients of BALB/c cells died from disseminated leishmaniasis within 90–110 days.) As in Fig. 1, the susceptibility profiles again correspond with those of the donor.

characteristic of the recipient, despite replacement of their macrophages by cells displaying donor phenotype *in vitro*¹¹, suggests a wider distribution of cell targets for this infection. In the case of avian leukosis virus, however, where the target is the B lymphocyte alone, transference of genetic resistance and susceptibility has been achieved in chickens by post-hatching exchange of semi-allogeneic bursal cells¹⁶. The conversion of resistance to *Salmonella typhimurium* into donor-type susceptibility has been achieved by establishing bone marrow chimaerism with the C57BL/10→(C57BL/10×A/J)_{F1} combination¹⁷, although the shift from susceptibility to resistance using the converse was detectable only in the early stage of infection and did not persist. The fact that *L. tropica* is an obligate macrophage parasite clearly contributes to the successful conversion of susceptibility into that of the haematopoietic cell donor in the present study. Although our data do not formally establish that the major gene expression is in macrophages rather than suppressor T-cell lineage, this does seem the more likely possibility by analogy with *L. donovani*, towards which isolated Kupffer cells behave *in vitro* as predicted by their strain of origin (J. Blackwell and D. J. Bradley, personal communication).

In conclusion, we report an example in which genetically determined susceptibility to a parasite infection is restricted to haematopoietic cell lineage, such that its replacement by radiation-induced chimaerism is accompanied by acquisition of the resistance/susceptibility phenotype of the donor.

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Recognition of cell types by axonal growth cones *in vitro*

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One of the most intriguing problems in neuroembryology is that of how growing axons find the correct way to their proper target cells. Several pathfinding mechanisms have been envisaged, including mechanical guidance through channels, guidance by diffusible substances and guidance by non-diffusible chemical cues expressed on cell surfaces and sensed by axonal growth cones. Here we describe experiments in which axons growing out from retinal explants are given the choice between two cell monolayers consisting of either retinal or tectal cells and we find that they grow preferentially over the surface covered with tectal cells. This preferential adhesion between retinal growth cones and cells originating from the *in vivo* target seems to be a property specific to the growth cone. In contrast, sorting experiments indicate that the perikarya of retinal cells adhere preferentially to retinal rather than tectal cells. It is likely that this adhesive property is due to recognition of cues expressed on the cell surfaces.

The experimental design is shown in Fig. 1. Axons originating from explants of chick retina grow through a zone where they are simultaneously exposed to two different cell types (retinal and tectal cells) and where they may 'choose' the more attractive one. A retinal explant is spread on a black membrane filter with the layer containing the ganglion cells facing up. The explant is stained with a fluorescent dye and placed upside down on the cell monolayer I. In growth medium, fluorescent axons grow from the explant towards a glass rod coated with monolayer II. Axons reaching the second monolayer have the choice between two cell types: either they decide to grow along monolayer I (Fig. 1, axon a) or along monolayer II (Fig. 1, axon b). This decision reveals their preference for a certain cell type. The ratio of fibres growing upwards along monolayer II and therefore out of the focal plane of monolayer I to that of the fibres which stay in that focal plane reflects the preference of the growth cones for the cell type of monolayer II.

To follow living axons growing in tissue or on a monolayer of cells, we have developed a technique using rhodamine–isothiocyanate (RITC) as a vital stain. Retinal explants stained with RITC and subsequently washed produce axons which remain well stained over 2 days. Figure 2 shows outgrowing fibres on an unstained monolayer of retinal cells. Outgrowing axons tend to keep their original direction over a long distance. The original direction of the fibres leaving the explant corresponds closely with the orientation of axonal growth within the retina *in vivo* where the axons are growing towards the optic fissure.

In control experiments both monolayers I and II consist of the same cell type. Our expectation is that half the axons reaching monolayer II on the glass rod should grow along monolayer I and the other half along monolayer II. However, as demonstrated in Table 1, only about 30% grow along monolayer II.

Table 1 Fraction of fibres growing along monolayer II

Monolayer I derived from	Monolayer II derived from		
	Retinal cells	Tectal cells	Telencephalic cells
Retinal cells	0.31 (158/352)	0.72 (1075/417)	0.39 (79/124)
Tectal cells	0.036 (63/1687)	0.34 (289/565)	0.16 (94/480)
Telencephalic cells	0.35 (46/86)	0.75 (130/43)	—

The total number of axons counted is shown in parentheses. The first number refers to axons growing along monolayer II, the second number refers to those which grow along monolayer I.

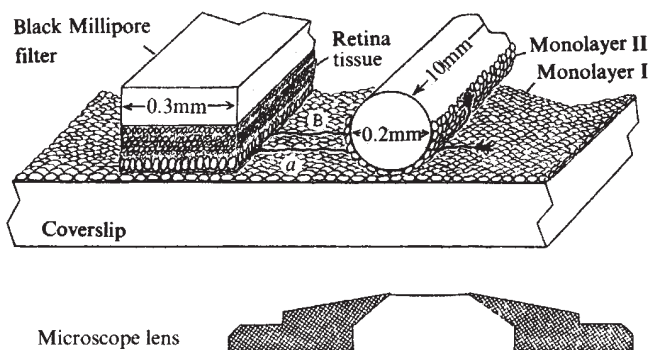


Fig. 1 Principle of experimental arrangement. The retina of a 6-day-old chick embryo was surgically isolated. A black membrane filter (Sartorius, SM 13006, $0.45\ \mu\text{m}$ pores) was soaked in Ca-Mg-free Hank's solution containing Con A ($0.1\ \text{mg ml}^{-1}$) for 1 h and subsequently washed with the same buffer. The retina was spread on the membrane filter in Ca-Mg-free Hank's solution. The membrane filter containing the retina preparation was placed briefly on a dry filter paper; thus, the retina becomes firmly attached to the membrane filter. This was then placed into a staining solution, prepared as follows: 1 mg of RITC is dissolved in $20\ \mu\text{l}$ dimethylsulphoxide; this solution is diluted 1:5,000 with vigorous shaking into Ca-Mg-free Hank's solution. After 15 min of incubation in staining solution at room temperature, the retina and the supporting membrane filter were washed three times with Hank's solution and then incubated in culture medium (Eagle's medium containing 10% calf serum and 2% chicken serum) for 1 h at 37°C . Then the supporting filter with the retina was again briefly placed on a filter paper to remove the liquid and chopped into strips 0.3 mm wide. These strips were placed on the monolayer as indicated in the figure. The layer of ganglion cells touches the monolayer. The strips were held in position by small stainless steel weights at the ends of the strips where they were not covered by retina. The monolayers were prepared as follows: retina, tectum or telencephalon of chick embryos was prepared and incubated for 10 min at room temperature and for another 10 min at 37°C in Hank's solution containing $1\text{--}2\ \text{mg ml}^{-1}$ trypsin. The tissue was then thoroughly washed, taken up in culture medium containing $25\ \mu\text{g ml}^{-1}$ DNase I at a concentration corresponding to $2\text{--}5 \times 10^8$ cells per ml, incubated for 5 min at 37°C and carefully dispersed by forcing the tissue twice through a capillary (10 cm long, 0.4 mm in diameter). This cell suspension was diluted with an equal volume of Ca-Mg-free Hank's solution and centrifuged for 10 min at 600 r.p.m. and 4°C . The sediment was taken up in Hank's solution at a concentration of 3×10^7 cells per ml. About 0.25 ml of the cell suspension was placed on top of a pretreated coverslip (20 mm in diameter) and the cells were allowed to settle at room temperature. After 15 min the coverslip was carefully rinsed with culture medium and kept in culture medium at 37°C until used for the experiment. The monolayers on the glass rods were prepared correspondingly. Pretreatment of coverslips and glass rods was necessary to achieve good cell adhesion. The silan treatment was done according to a method described by Gottlieb⁴. Before being used for production of monolayers, the derivatized glass was cleaned by wiping with cotton, then incubated in a Con A solution ($1\ \text{mg ml}^{-1}$ Con A in Hank's solution) at room temperature for 1 h and finally rinsed in Hank's solution. Microscopic observation was done at ~24 h after explantation, as described in Fig. 2 legend. Whether an axon is rising or staying on monolayer I was determined for each axon by changing the focal plane of observation.

The tendency of axons to remain on the coverslip monolayer may reflect a tendency to grow in straight rather than curved paths, or incomplete contact between the monolayers.

In test experiments, one of the monolayers was prepared from retinal cells, the other from tectal cells. In all experiments retinal axons show a strong preference for cells derived from the tectum, their *in vivo* target. The preference is more pronounced if monolayer I contains tectal cells (Table 1). In 15 separate experiments, 72% of a total of 1,492 fibres preferred tectal cells on the glass rod to retinal cells on the coverslip, whereas only 4% of 1,416 fibres grew to the glass rod when it was covered with retinal cells and the coverslip contained the tectal cells. There are slight variations between experiments but in all 15 in which the glass rod carried the tectal cells, appreciably more than 50% of the axons grew along the glass rod; in none of the experiments with tectal cells on the coverslip did more than 10% of the axons grow along the glass rod carrying retinal cells. Telencephalon monolayers are no more attractive for retinal axons than retinal monolayers, and much less attractive than tectal monolayers (Table 1).

It should be mentioned that the monolayers are contiguous, that the axons grow exclusively on cell monolayers and that they are completely unable to use pretreated glass covered with concanavalin A (Con A) as a substratum. At the interface of monolayer I and II, neither branching nor growing back on monolayer II has been observed.

It would be interesting to know whether axons arising from a certain position within the retina show a preference for tectal cells from a corresponding tectal area in this *in vitro* system. Experiments on antero-posterior positional effects are in progress.

Our experiments show that the growth cones of retinal axons have a clear preference for tectal cells. Is this preference a general surface property of retinal cells or is it specific to their outgrowing axons? This question can be answered by sorting experiments of the kind extensively used by Moscona¹. Single-cell suspensions of retinal and tectal cells are mixed and allowed to form aggregates. If these aggregates are incubated for 24 h the cells sort out in such a way that the core preferentially

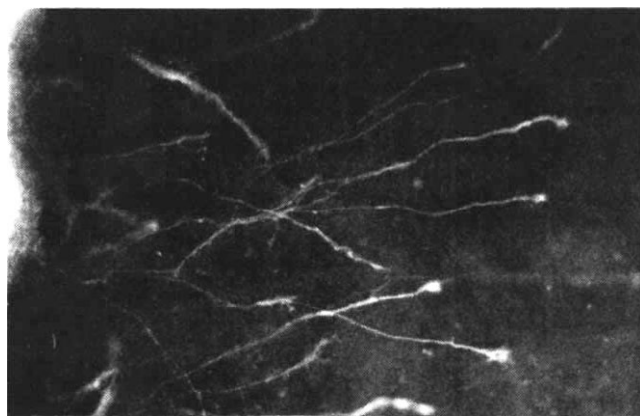


Fig. 2 Fluorescent retinal axons growing on a monolayer of retinal cells. The edge of the stained explant is at the left. Staining of explant and preparation of monolayer are described in Fig. 1 legend. The microscopic observation is done with epifluorescence at a wavelength $>590\ \text{nm}$ in an inverted Zeiss microscope with an image-intensifying TV camera (Siemens). The incident light range is 510–560 nm. With this technique the axons can easily be detected on a monolayer of cells.

contains retinal cells, whereas the periphery consists preferentially of cells derived from the tectum. This sorting out can be observed in a fluorescence microscope if, for example, the retinal and tectal cells have been stained before mixing with RITC and fluorescein isothiocyanate (FITC), respectively. Pictures of two typical aggregates after sorting are shown in Fig. 3. The fluorescence of tectal cells is seen in Fig. 3a, that of retinal cells in Fig. 3b. Black-and-white pictures of the whole aggregates taken in one plane of focus inadequately demonstrate the clear sorting out of the two cell types, whereas direct observation of the stained cells in various focal planes show sorting out very clearly. In experiments with tectal and retinal cells derived from embryos of various ages (6, 7, 9 days), retinal cells are always in the centre of the aggregates and tectal cells always in the outer shell. According to Steinberg², sorting experiments of this kind reveal a hierarchy of cell adhesiveness; those cells in the middle adhere more tightly to each other than to the cells of the outer shell. This indicates that for cell perikarya retina–retina adhesion is stronger than retina–tectum adhesion, in contrast to the adhesion of the retinal growth cones which, in spite of their retinal origin, adhere preferentially to tectal cells.

The combination of axon choice and cell-sorting experiments suggests that growth cones have adhesive properties which differ from the adhesive properties of the perikarya from which they

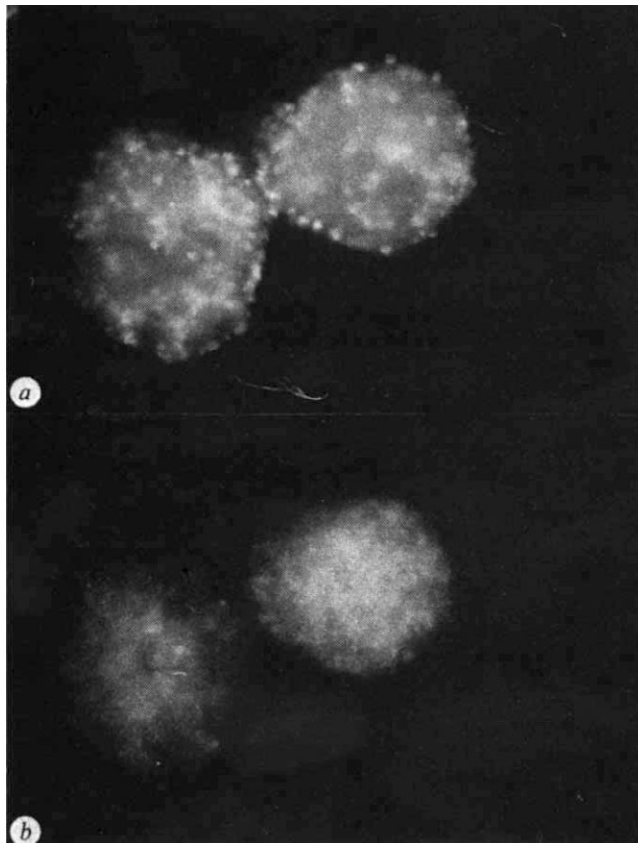


Fig. 3 Sorting out of tectal and retinal cells. Tectal and retinal cells were labelled with RITC and FITC, respectively, as described by Burt and Gierer⁵, and taken up in Eagle's medium containing 20% calf serum, mixed at a concentration of about 10^7 cells ml^{-1} for each cell type. They were incubated on a gyratory shaker in an Erlenmeyer flask (25 ml) with 60 r.p.m. at 37°C for 18–30 h. *a*, Fluorescence of tectal cells was observed at wavelengths >590 nm with an illumination at 546 nm. *b*, Fluorescence of retinal cells was observed at wavelengths of 520–560 nm with an illumination of 450–490 nm.

originate. However, remember that sorting experiments are concerned with a mixture of all types of retinal and tectal cells and not with a pure population of retinal ganglion cells—probably the only origin of all fibres observed—and tectal target cells.

It has long been known that axonal growth is very dependent on the material offered as substratum (see, for example, ref. 3). Our experiments show that the growth cones of retinal cells can discriminate *in vitro* between various neural cell types and this capability may be used for *in vitro* axonal guidance. It remains to be shown that the guidance of axons *in vitro* corresponds with the axonal guidance in a developing brain. We believe that the present experiments strongly support the idea that growth of axons along specific pathways *in vivo* may be mediated by characteristic affinities of growth cones for specific cell surfaces. The system we describe lends itself to biochemical analysis of the specific recognition of cell types by growth cones.

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Cross-reactivity for different type A influenza viruses of a cloned T-killer cell line

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Spleen cytotoxic T cells killing influenza virus-infected target cells are cross-reactive for the different type A influenza viruses, in contrast to the circulating antibodies, which show fine specificity for each A virus subtype variant^{1,2}. This finding has raised the question of whether a single T cell can recognize cells infected with all the type A viruses. T-killer cell lines with specificity for alloantigens and the male Y antigen can be selected by means of growth factors present in the supernatant of T cells stimulated with concanavalin A (refs 3–7). We report here that we have been able to establish clones of mouse T cells killing target cells infected with influenza virus. Our cell line maintains the same specificity as the heterogeneous spleen cell population from infected mice, in as far as the T-killer cells are specific for A influenza virus, but do not discriminate between the different type A viruses. The cell line maintains H-2 restriction and does not kill cells infected with B influenza virus. The cells grow in the presence of T-cell growth factor and do not require antigen for growth although they maintain their receptors for type A virus. They can also be stimulated by irradiated T-helper cells from mice primed by type A influenza infection in the presence of type A virus-infected cells.

The type A influenza specific killer cells were selected by methods similar to those described for allogeneic killer cell lines^{3–8}. Briefly, BALB/c donor mice were primed by infection with A/X-31 (H3N2, WHO nomenclature⁹) influenza virus and cytotoxic T cells (CTLs) were generated by culture of the primed spleen cells with A/X-31-infected lymphoblasts¹. The cells were restimulated in the same way every 10–12 days (7.8×10^5 cells stimulated with 1.5×10^5 ml irradiated infected lymphoblasts) and after 2 months the cells grew well in the absence of antigen in RPMI 1640 medium containing 10% fetal calf serum and 30% rat cell supernatant (RS). RS supernatant supporting the growth of the T cells was prepared by stimulating 3×10^5 rat spleen cells per ml with $3 \mu\text{g ml}^{-1}$ concanavalin A for 48 h (refs 3–8). The presence of T-killer cells were ascertained by assaying lysis of ⁵¹Cr-labelled P815 (H-2^d) mastocytoma cells infected with type A influenza virus, or non-histocompatible infected target cells. After 4 weeks the cells could be cloned on layers of syngeneic mouse peritoneal macrophages (irradiated with 1,600 rad) using two to five cells per well in 0.1 ml in a Linbro microplate. One of the clones (L4) was highly cytotoxic for influenza-infected cells at low killer/target cell ratios. On recloning the L4 killer cells, all the subclones lysed A/X-31-infected target cells (Table 1). The clones with the highest activity were grown and tested for their specificity. Clone L4 was found to show the same specificity as the mixed population of T-killer cells generated *in vitro* by culture of primed spleen cells with infected stimulators (Table 2). The L4 killer cells do not discriminate between the different subtypes and variants of influenza virus A. Target cells infected with any of the type A viruses are lysed equally well (USSR/70) (H1N1), Jap 305 (H2N2) or X-31 (H3N2), whereas cells infected with B/HK influenza virus, or H-2^k-infected target cells are not attacked. Thus, H-2 restriction is preserved.

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Table 1 Cytotoxicity of clone L4 and its subclones

K/T	% Target cell lysis		
	5/1	4/1	3/1
L4			26.5
L4/1		62.9	
L4/2		40.1	
L4/5		44.2	
L4/9	33.9		
L4/11			31.0
L4/13			35.9
L4/14		29.2	

⁵¹Cr-labelled P815 target cells (H-2^d) were infected with A/X-31 virus for 1 h. Following the addition of killer cells, lysis of 2×10^4 target cells was assayed after 6 h by estimating the amount of ⁵¹Cr released by the killer cells¹⁷. Control spontaneous release was 18%. Target cell lysis was expressed as %⁵¹Cr released = c.p.m. (experimental) - c.p.m. (spontaneous release)/c.p.m. (total) - c.p.m. (spontaneous release). K/T, killer/target cell ratio.

With time of culture, cytotoxicity and the rate of growth of L4 clones seem to decline. However, we have been able to grow the cells from frozen stock and to reclone on irradiated macrophages and have maintained highly cytotoxic clones for 8 months.

Although the established L4 cell line does not require antigen for its growth, because growth is supported by nonspecific and non-H-2- or strain-restricted growth factors produced by activated T cells, the cells clearly preserve their receptors for antigen recognition. First, L4 cells retain their killer specificity for type A influenza viruses, and second, 20% of the continuously growing T cells form clusters with P815 cells infected with type A virus but not with cells infected with influenza B virus (experiments with W. A. Taylor, using the method of Berke *et al.*¹⁰).

Growth and killer activity of L4 clones can also be induced in the presence of macrophages by stimulation with infected stimulator cells (irradiated spleen) and irradiated T-helper cells¹¹ from mice primed by infection with type A virus (Table 3). The L4 killer cells die in the absence of T-cell growth factor (RS) or irradiated T-helper cells plus antigen. The requirement for T-cell growth factor on its own distinguishes the T-cell clones from memory precursors of cytotoxic T cells in mice primed by infection. We find that the memory cells require antigen and T-cell growth factors for the generation of cytotoxic cells (B.A.A., unpublished results). This indicates that the continuously growing cells are at the last stage of differentiation. Fluorescent staining with monoclonal rat antibodies showed

Table 2 Specificity of L4 killer cells, H-2 restriction and cross-reactivity between different type A influenza viruses

H-2	Target cells Influenza infection	K/T	% ⁵¹ Cr release			
			10	5	2.5	1.2
d	A/X-31 (H3N2)*		60.4	54.7	39.8	27.7
d	A/Jap (H2N2)		71.6	62.9	49.1	41.2
d	A/USSR (H1N1)		79.6	65.8	59.9	54.9
d	B/HK		6.5	4.7	1.9	1.6
d	Uninfected		4.7	2.1	1.1	1.3
k	A/X-31 (H3N2)		7.5	7.7	4.5	0

The L4 cells were derived from mice primed with A/X-31 virus and induced *in vitro* with the same virus. Target cells were labelled with ⁵¹Cr and infected with influenza virus¹⁷. H-2^b target cells were CBA lymphoblasts induced with lipopolysaccharide and infected with A/X-31 virus. Such target cells are lysed by CBA T-killer cells. H-2^d target cells were similarly infected P815 mastocytoma cells. ⁵¹Cr-release assay was as in Table 1.

* H = viral haemagglutinin, WHO nomenclature⁹; N = viral neuraminidase.

Table 3 Cloned T-killer cells respond to virus-stimulated T-helper cells

Clone L4/2 (cells $\times 10^{-4}$ per well)	10 ⁵ Stimulator cells	4 $\times 10^4$ Macrophages	1.5 $\times 10^5$ T-helper cells +	% Target cell lysis
2.5	+	+	+	10.4 (± 1.1)
5	+	+	+	19 (± 0.6)
5	-	+	-	0
5	-	+	+	0
-	+	+	+	0
2.5	-	+	-	0
5	-	+	-	0
5	+	+	-	0

Stimulator cells are normal spleen cells irradiated (1,200 rad) and infected with A/X-31 influenza virus. Peritoneal exudate macrophages were seeded into microwells and irradiated at 1,600 rad before addition of the T-killer clone. +T-helper cells were spleen cells irradiated at 600 rad (ref. 11) and derived from mice primed intranasally by infection with A/X-31 virus 2 months previously. Target cell lysis on day 4 was assayed on 10^4 A/X-31 infected P815 mastocytoma cells, labelled with ⁵¹Cr and added to the microwells after centrifuging the plate and removing the culture medium. Values for mean lysis by 6-12 culture wells are given ($\pm$ s.e.).

that the L4 killer cells bear the Lyt 2 marker, but are Lyt 1 negative.

We have not been able to convert the L4 cytotoxic cells into memory cells by transferring them into syngeneic mice and challenging with influenza virus. However, we have been able to protect mice from death following lethal infection with A/X-31 virus. BALB/c mice were irradiated at 450 rad (Co source) and infected with A/X-31 influenza intranasally (25-50 haemagglutinin units in 100 μ l of 1/100 allantoic fluid). One day later, the mice received normal spleen cells, or our continuously growing cytotoxic T cells. Mice receiving no cells, or 3×10^6 normal spleen cells intravenously died by day 17 or earlier, whereas the mice which had received 3×10^6 L4 cytotoxic cells survived for more than 4 weeks. The cloned cells therefore show protective activity *in vivo* as do primed normal T cells restricted at the K and D end of H-2 (ref. 12).

It is not certain which viral protein is being recognized by A influenza-specific T-killer cells, but our killer cell line should help resolve this question. The internal matrix protein shared between the different type A viruses was previously thought to be recognized by cross-reactive T cells^{13,14}; however, recent data indicate that killer T cells recognize a region of the haemagglutinin molecule homologous in all type A influenza viruses¹⁵⁻¹⁷.

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Reversible heat activation of alkaline phosphatase of *Dictyostelium discoideum* and its developmental implication

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The activities of some enzymes increase during the development of the cellular slime mould, *Dictyostelium discoideum*. Because optimal specific activity is attained by each enzyme at a specific developmental stage¹, development can be followed as a function of enzyme activity. The activity of one of these enzymes, alkaline phosphatase, increases markedly during the late stages of development, making it a potentially useful marker for terminal differentiation^{2,3}. It has been suggested that this increase in activity is due to *de novo* enzyme synthesis because the increase in activity does not occur in the presence of inhibitors of RNA and protein synthesis². However, we present evidence here of reversible heat activation of membrane-bound alkaline phosphatase which suggests that the increase in alkaline phosphatase activity may be due to an unmasking of pre-existing enzyme by a novel membrane regulatory mechanism.

Alkaline phosphatase is firmly membrane bound, and evidence suggests that it is located on both the plasma membrane³⁻⁵ and the internal contractile vacuole membrane⁶. We found that there was a considerable decrease in alkaline phosphatase activity if a crude membrane preparation from vegetative-phase cells was stored at 4 °C. Activity was restored, however, if the membrane preparation was incubated at room temperature, and a dramatic increase in activity was observed if the enzyme was incubated at elevated temperatures. With increasing temperature the rate and amount of the enzyme activation increased (Fig. 1), reaching a maximum at 50 °C. Although not shown in

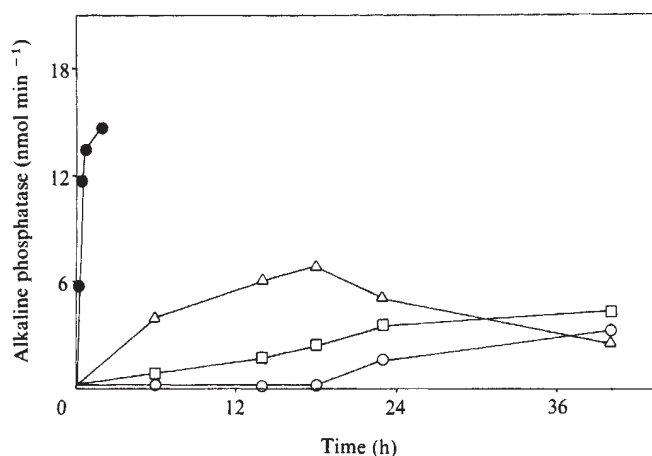


Fig. 1 The effect of preincubation temperature on the activity of membrane-bound alkaline phosphatase. Crude membranes of vegetative cells of *D. discoideum*, strain Ax-2, were prepared as described previously⁸, resuspended in 5 mM Tris-Cl, pH 7.4, at 4 mg ml⁻¹ and incubated at 13.5 °C (○), 21 °C (□), 33.5 °C (△) or 50 °C (●). After the indicated periods of incubation, 0.1-ml aliquots of the membrane were assayed for alkaline phosphatase activity at 30 °C in a total assay volume of 1.0 ml as described previously³, with the addition of 30 mM NaF to inhibit acid phosphatase.

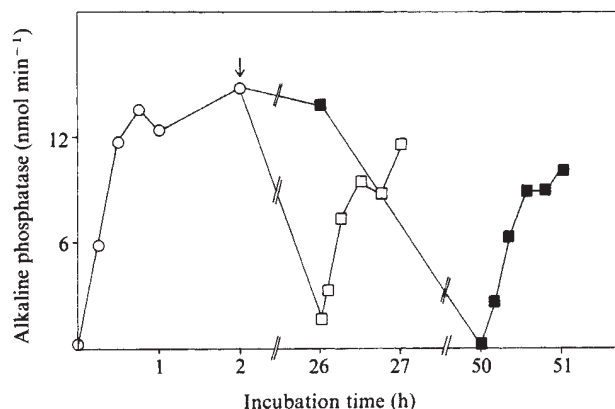


Fig. 2 The reversible heat activation of alkaline phosphatase. Crude membrane preparations of vegetative cells were incubated at 50 °C and aliquots were removed at the indicated time points (○) and assayed for alkaline phosphatase activity at 30 °C. After 2 h of incubation at 50 °C (at the arrow) the preparation was divided into two and one portion (□) was incubated for 24 h at 0 °C followed by 1 h at 50 °C. At the indicated time points (□), aliquots were removed and assayed for alkaline phosphatase at 30 °C. The second sample (■) was rapidly cooled to -70 °C, maintained at -70 °C for 24 h and then incubated at 0 °C for 24 h, followed by 1 h at 50 °C. At the indicated time points (■), aliquots were assayed for alkaline phosphatase activity at 30 °C.

this experiment, activity at 50 °C is stable for up to 6 h. Incubation at 60 °C led to variable results (data not shown), whereas incubation at 70 °C resulted in a total inactivation of the enzyme.

This heat activation is totally reversible. A membrane preparation was heated at 50 °C for 2 h, and alkaline phosphatase activity increased markedly (Fig. 2). The preparation was then divided and one portion stored at 0 °C for 24 h, whereas the other was rapidly frozen in acetone/dry ice and stored at -70 °C for 24 h. The membrane fraction stored at 0 °C lost considerable alkaline phosphatase activity, but when reheated at 50 °C, activity was rapidly restored (Fig. 2). This heat activation-cold inactivation cycle could be repeated three times (data not shown) indicating that the phenomenon was totally reversible. The sample stored at -70 °C retained the elevated heat-activated level (Fig. 2), but when transferred to 0 °C, the activity declined markedly over a 24-h period. The enzyme could be reactivated by incubating at 50 °C.

Thus, membrane-bound alkaline phosphatase activity is markedly dependent on the temperature of storage. The mechanism of the reversible temperature-dependent activation is not known, but the factor(s) involved in the phenomenon are tightly membrane bound, as even after recentrifugation at 100,000g and resuspension in fresh buffer, both heat-activated and cold-inactivated membranes retained their capacity for the reversible activation or inactivation of the enzyme.

Table 1 Comparison of the effects of preincubation at 50 °C and 0 °C on the alkaline phosphatase activity of intact membrane and butanol-extracted preparations

Preparation	No treatment	Preincubated at 50 °C for 60 min	Preincubated at 0 °C for 24 h
	Specific activity of alkaline phosphatase (nmol min ⁻¹ per mg protein)		
Intact membrane	9.2	74.0	3.1
Butanol extract*	105.0	56.0	96.0

* The butanol-extracted preparation was prepared by briefly vortexing a mixture of 0.4 ml butanol and 1.0 ml of a suspension of crude membranes in 5 mM Tris-Cl, pH 7.4 (10 mg protein ml⁻¹). The aqueous phase was removed carefully, dialysed for 24 h against 0.01 M Tris-Cl, pH 7.4, and assayed for alkaline phosphatase activity at 30 °C after the indicated preincubation conditions. Over 70% of the activated membrane-bound alkaline phosphatase is solubilized by this procedure.

To investigate this phenomenon further, alkaline phosphatase activity was solubilized from the membrane by butanol treatment. Following butanol extraction the alkaline phosphatase activity of the aqueous phase was elevated to a level slightly higher than that observed following heat treatment (Table 1). When this solubilized enzyme was incubated at 0 °C for 24 h there was no loss in activity, whereas incubation at 50 °C for 60 min caused a reduction in activity, characteristics quite different from those of the enzyme of the intact membrane (Table 1). Thus, maintenance of membrane integrity seems to be essential for the expression of the reversible heat activation-cold inactivation of the enzyme.

The alkaline phosphatase activity of freshly prepared membranes from cells at later stages of development is considerably higher than that of vegetative cells, reaching peak levels at the culminating stages of development (Table 2). The activity of culminating cell membranes is not markedly responsive to heat activation or cold inactivation; there is no decrease in activity on storage at 0 °C for 24 h, and only a slight elevation on incubation at 50 °C for 1 h (Table 2). Alkaline phosphatase from pseudoplasmodial-stage cells is partially responsive to the heat activation phenomenon (Table 2). There is a slight cold-activation effect in pseudoplasmodial membranes but at present this phenomenon is not understood. As the peak activities of alkaline phosphatase in culminating membranes is of the same order of magnitude as the maximum activated levels of vegetative membranes, clearly vegetative-phase cells express only a small proportion of their potential alkaline phosphatase activity, whereas the culminating-phase cells express most of theirs. There is a twofold increase in the heat-treated levels of activity in culminating cells compared with vegetative cells (Table 2). This may indicate that heat treatment does not fully unmask the vegetative activity. Alternatively, as the amount of protein per cell decreases during differentiation⁷, the amount of membrane protein per cell probably decreases concomitantly, and the twofold increase in the specific activity of a membrane-bound enzyme would reflect no change in the amount of enzyme per cell. We have not investigated this possibility further due to the difficulty of determining accurately the yields of differentiating cells.

Table 2 Comparison of the effects of preincubation of 0 °C and 50 °C on the alkaline phosphatase activity of membranes prepared from Ax-2 cells at various stages of development

Developmental stage	Developmental time (h)	Freshly prepared membranes	Preincubated at 0 °C for 24 h	Preincubated at 50 °C for 1 h
Alkaline phosphatase specific activity (nmol min ⁻¹ mg ⁻¹)				
Vegetative	0	9.2	3.1	74.0
Pseudoplasmodium	22	43.0	71.0	105.0
Preculmination	30	108.0	105.0	139.0
Late culmination	34	105.0	92.5	133.0

It should be emphasized that the alkaline phosphatase activity of vegetative cells is the same, whether the membranes are prepared at 4 °C or 22 °C (data not shown), indicating that the low activity of freshly prepared vegetative membranes is not due to the low temperature of the preparative procedure⁸. We believe, therefore, that the apparent increase in alkaline phosphatase activity is due to unmasking of existing enzyme rather than to an increase in amount of enzyme. McCleod and Loomis⁹ have recently presented evidence that the vegetative and developmentally determined enzymes are products of the same gene, a finding not inconsistent with the above suggestion.

This lack of correlation between the observed level of the enzyme and the amount of enzyme protein has also been reported during *D. discoideum* development (for example, see ref. 10). However, this is the first instance of an apparent unmasking of a presumptive developmentally regulated enzyme by heat treatment.

Although there is probably no direct physiological significance to the reversible heat activation of the membrane-bound alkaline phosphatase, this phenomenon apparently simulates the effect of a plasma membrane modification that occurs during development. This could involve the removal of a low-molecular-weight effector molecule or the dissociation of inactive protein aggregates within the plane of the membrane. We are investigating these possibilities.

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SV40 large T shares an antigenic determinant with a cellular protein of molecular weight 68,000

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The large T protein coded by the early region of simian virus 40 (SV40) is involved in the induction and maintenance of cell transformation¹. It is not clear which properties of T are important in causing the transformation, since the protein is multifunctional²⁻⁹. To clarify the action of T proteins in transformation, we have prepared several monoclonal antibodies directed against different regions of the T molecule. One of these antibodies, DL 3C4, recognizes an antigenic determinant on T that is also present on a host cell protein of molecular weight 68,000. This cross-reactive 68K protein is located within the nucleus of all mammalian cell types examined and has a characteristic granular distribution as shown by immunofluorescence. The antigenic determinant recognized by DL 3C4 is resistant to denaturation. Studies on Adeno-SV40 hybrid viruses show that the antigenic site on T is coded for by sequences located between 0.44 and 0.29 on the SV40 map.

The cell line producing the monoclonal antibody DL 3C4 was derived from a fusion between the HAT (hypoxanthine, aminopterin and thymidine)-sensitive myeloma cell line Sp2/O-Ag14 (refs 10-12) and spleen cells from a BALB/c mouse which had been hyperimmunized with the T antigen-related protein purified from Ad2⁺ D2-infected HeLa cells⁵. Ten days after fusion cell culture supernatants were screened, using an indirect immunofluorescence test, for antibodies capable of staining the nuclei of SV40-transformed cells (SV80). The supernatants from 11 out of 96 wells were positive by this test and the cells from these wells were cloned using a soft-agarose method. (A detailed description of the methods will be published elsewhere, D. L., in preparation.)

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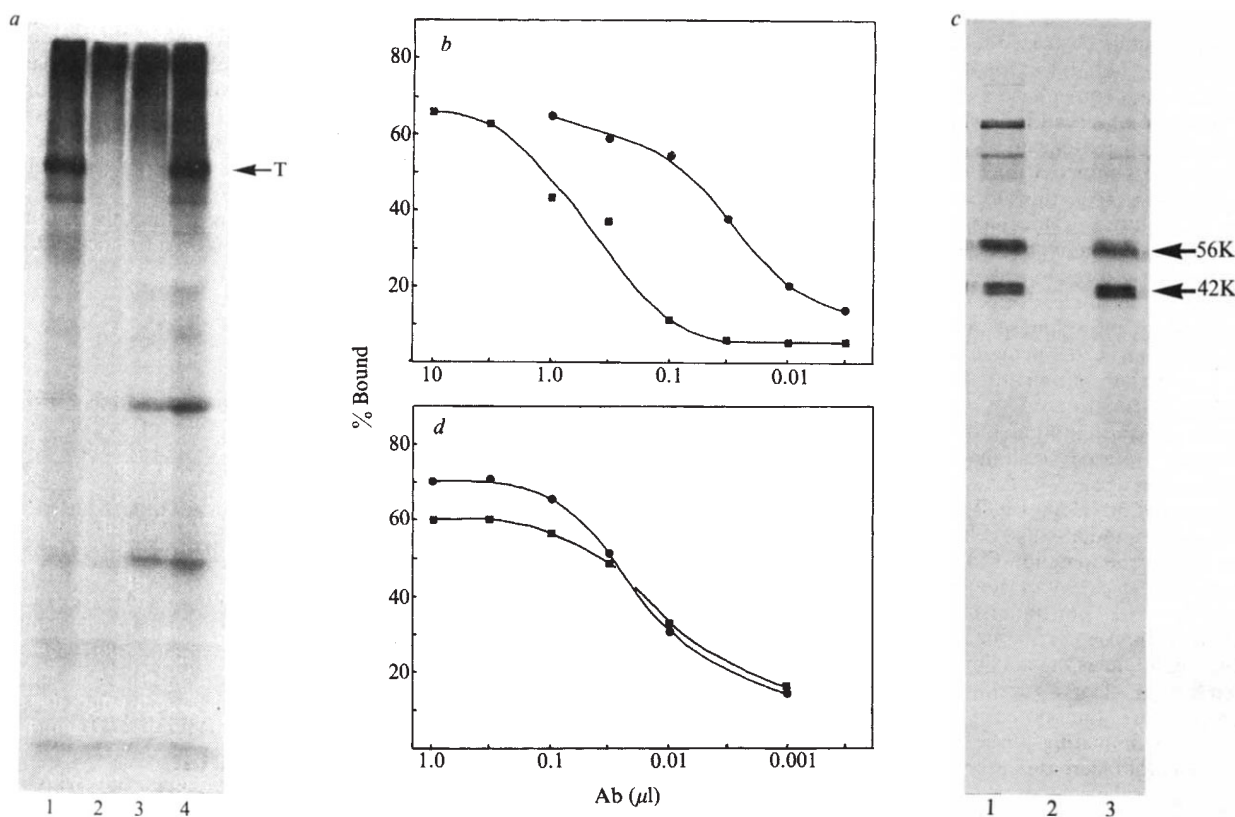


Fig. 1 Monoclonal antibody 3C4 binds to a specific region of SV40 large T antigen. *a*, SV80 cells, in a 60-mm Petri dish, were labelled for 1 h in phosphate minus E4 medium with 1 mCi of ^{32}P and the labelled proteins extracted in 1 ml of 1% NP40, 120 mM NaCl, 50 mM Tris, pH 8.0. 100 μl of the extract were incubated with (1) 10 μl hamster anti-tumour serum, (2) 10 μl normal hamster serum, (3) 50 μl E4 medium and (4) 50 μl supernatant E4 medium from a culture of 3C4 cells. After 1 h at room temperature 20 μl of rabbit anti-mouse immunoglobulin serum (Rb α Mlg) were added to tubes (3) and (4) as 3C4 does not bind to protein A directly and then 1 h later 40 μl of a 10% suspension of fixed *Staphylococcus aureus* Cowan 1 (ref. 21) were added. The bacteria were collected by centrifugation, washed four times in NET buffer²¹ and the adsorbed immune complexes eluted by boiling in 50 μl of SDS-gel sample buffer. The eluted proteins were analysed on a 15% polyacrylamide slab gel²⁰ and the labelled proteins detected by autoradiography of the stained fixed and dried gel. (The minor band below large T is a 73K breakdown product of T). *b*, Purified ^{35}S -labelled large T antigen was isolated by immunoprecipitation and preparative SDS-gel electrophoresis from lysates of ^{35}S -labelled SV40-infected CV1 cells as before¹³. It was then used to titrate the relative T-binding capacity of a rabbit antibody to large T (■) and of the monoclonal antibody 3C4 (●). The monoclonal antibody was used as the ascites fluid from a BALB/c mouse which had been injected intraperitoneally 10 days earlier with 10^7 3C4 cells. The radioimmunoassay was as described previously except in the case of the monoclonal antibody 20 μl of Rb α Mlg were added, 1 h before the addition of 50 μl SAC. Input was 1,000 c.p.m. of T. *c*, A 60-mm Petri dish contained HeLa cells, labelled with 500 μCi of ^{35}S -methionine in met⁻E4 medium 30 h after infection with Ad2⁺ND2 virus. After labelling for 1 h the labelled proteins were extracted as described in *a* and immunoprecipitated with (1) 10 μl rabbit anti-T serum, (2) 20 μl Rb α Mlg, and (3) 0.1 μl 3C4 ascites fluid + 20 μl Rb α Mlg. The antibody-bound proteins were collected and analysed as in *a* except that a 10% SDS-polyacrylamide gel was used. *d*, Purified ^{35}S -labelled 56K and 42K T-related proteins were isolated from extracts of ^{35}S -labelled Ad2⁺ND2-infected HeLa cells as described for large T in *b*. The isolated proteins were then individually used in the radioimmunoassay to titrate the 3C4 ascites fluid binding to (■), the 56K protein (input 2,070 c.p.m.) and to (●), the 42K protein (input 2,360 c.p.m.).

The hybridoma cell line DL 3C4 has been cloned four times and grows well in culture. When injected into pristane-primed mice it induces an ascites tumour and the ascites fluid contains extremely high titres of the antibody ($>1/10^4$ in the immunofluorescence test). The antibody synthesized by 3C4 specifically immunoprecipitates T antigen from lysates of ^{32}P -labelled SV80 cells (Fig. 1*a*) and confirmation of its specificity for large T was obtained by radioimmunoassay¹³. The ascites fluid and supernatant tissue culture media of 3C4 cells were able to bind specifically pure ^{35}S -labelled T antigen. (Isolated from ^{35}S -methionine-labelled SV40-infected monkey cells by immunoprecipitation with a defined anti-T serum¹³ followed by SDS-gel electrophoresis.) Figure 1*b* shows the titration 3C4 ascites fluid in such a radioimmunoassay. The monoclonal antibody could also immunoprecipitate the T-related proteins of molecular weights 56,000 and 42,000 present in extracts of Ad2⁺ND2-infected HeLa cells (Fig. 1*c*). Furthermore the antibody bound specifically to both polypeptides after they had been isolated by immunoprecipitation and elution from SDS gels (Fig. 1*d*). These experiments show that the 3C4 antibody

recognizes a determinant on T which is coded by the SV40 DNA present in the Ad2⁺ND2 virus (0.44–0.11 map units)¹⁴ and which is resistant to the denaturing effects of SDS-gel electrophoresis. The 3C4 antibody did not immunoprecipitate or bind to the 30,000-molecular weight T-related protein synthesized in Ad2⁺ND1-infected cells (data not shown) and it is reasonable to assume that the determinant is therefore not coded by the SV40 DNA in this virus (0.29–0.11 map units)¹⁵. This approximately localizes the region coding for the determinant to sequences between 0.44 and 0.29 on the SV40 map. The determinant must be present on the majority of T-antigen molecules as the monoclonal antibody can bind the same proportion of the isolated ^{35}S -labelled T antigens as a variety of other anti-T sera (Fig. 1).

Having observed the specificity of 3C4 for a particular region of T antigen, it was surprising to discover that the antibody gave a positive specific nuclear fluorescent staining pattern on a wide variety of different cell types that do not contain SV40 DNA or the SV40 T antigen. Figure 2 shows examples of this staining pattern on two different mammalian cell lines: KA31 cells are RNA-virus transformed murine fibroblast cells and PtK1 cells

are untransformed kidney epithelium cells from the marsupial *Potorous tridactylis*¹⁶. In both lines, especially PtKI cells, the staining pattern is clearly nuclear and strikingly granular. Various cell lines, including cells of human, monkey and rat origin, all show the positive nuclear staining pattern, so the determinant being recognized is clearly highly conserved in the mammals. None of the other anti-T monoclonal antibodies examined, nor a variety of conventional anti-T sera, show such positive reactions on these cells, so the effect seems to be restricted to the DL 3C4 monoclonal antibody.

The species recognized by 3C4 in one of these SV40 T antigen-negative cell lines was identified using the immunoprecipitation technique described by Burridge¹⁷. A single protein of molecular weight 68,000 is detected in extracts of mouse L cells (Fig. 3). Attempts to identify this protein by immunoprecipitation of conventional NP40 lysates of these cells were only partially effective. The protein is present in low concentration and poorly solubilized by buffers that effectively extract the viral T antigen from SV40-infected or -transformed cells, most of the antigen remaining in the insoluble pellet.

One hypothesis to explain the apparent dual specificity of 3C4 for the virally coded SV40 T antigen and the 68K host cell protein is that these two proteins share a common structural

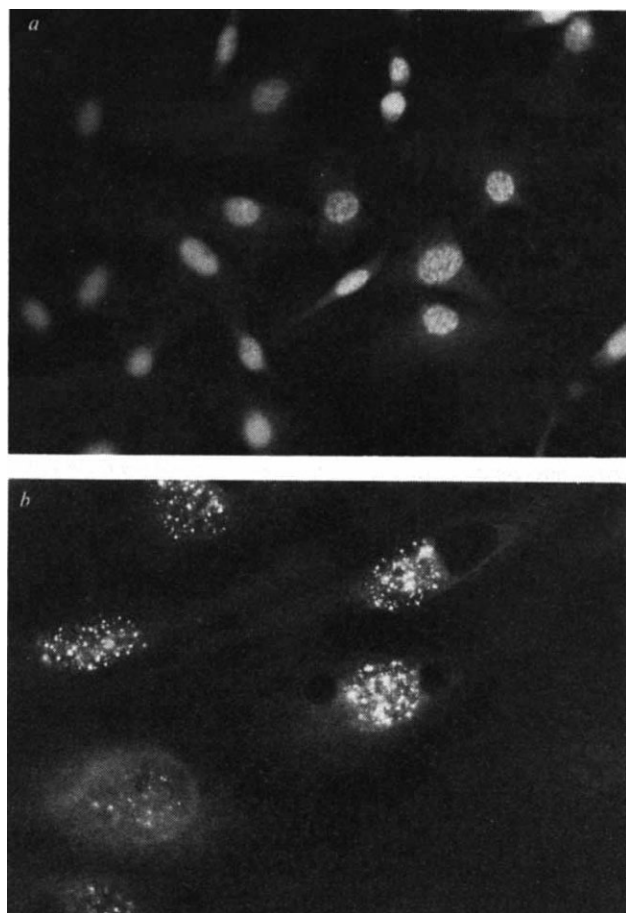


Fig. 2 Detection of a T-related protein in the nuclei of cells that do not contain SV40 DNA. Cells for immunofluorescence were grown on glass coverslips, washed in PBS, fixed in a 1:1 mixture of acetone and methanol at room temperature for 2 min, then overlaid with a 1/50 dilution of 3C4 ascites fluid. After 20 min of incubation the coverslips were washed in phosphate-buffered saline (PBS) and then overlaid with a 1/30 dilution of rhodamine-conjugated rabbit antibody to mouse immunoglobulin (Cappel). After a 20-min incubation the coverslips were washed in PBS and then with distilled water before mounting in gelvatol (Monsanto Chemicals) on clean glass slides. The coverslips were examined under a fluorescence microscope. *a*, Staining of KA31 cells. *b*, Staining pattern of PtK-1 cells.

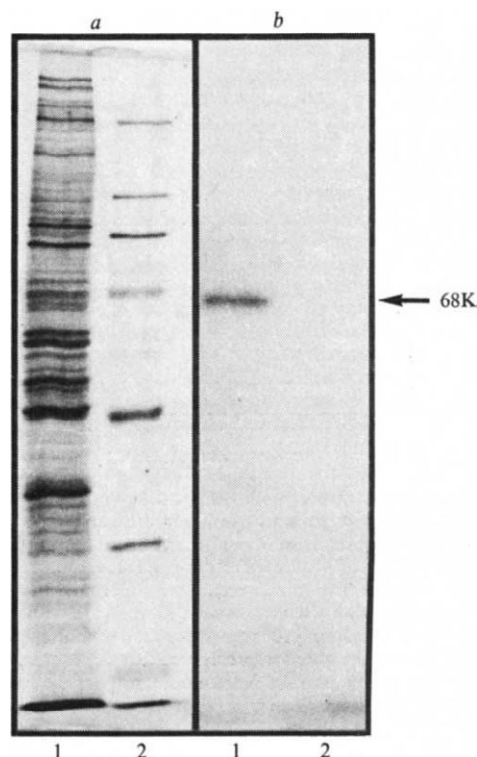


Fig. 3 Immunoautoradiographic detection of the protein bound by 3C4 in cells that do not contain T-antigen. A complete nuclease-treated extract of mouse L cells, solubilized in sample buffer, was electrophoresed on a 10% SDS-polyacrylamide gel. Track 1, 40 µl L cell extract (2×10^5 cells). Track 2, Marker proteins: 200K, 130K, 94K, 45K, 30K, 21K. *a*, Shows the gel stained with Coomassie blue. In *b*, the same gel strip was fixed lightly, equilibrated, and then overlaid first with monoclonal antibody DL 3C4 (as tissue culture supernatant) for 4 h and then after extensive washing in Tris-buffered saline it was overlaid with 125 I-labelled rabbit anti-mouse immunoglobulin antibody for 3 h. After further extensive washing, the gel strip was stained dried and autoradiographed. *b*, Shows the result of a 46-h exposure to Kodak XR-1 film with a lightening-plus intensification screen at -70°C . The method used is precisely that of Burridge¹⁷.

element that forms the antigenic site recognized by 3C4 antibody. That extracts of cells that did not contain T could, nevertheless, inhibit the binding of 3C4 to purified ^{35}S -labelled T antigen (Fig. 4) supports this hypothesis. This result forms the basis of a radioimmunoassay for the host cell protein and eliminates a trivial explanation for the pattern of 3C4 reactivity—that the cell line produces two different antibodies, one specific for T and one specific for the 68K protein. Possibly some conventional anti-T sera have arisen that recognize this cross-reactive 68K protein. Indeed Renart *et al.*¹⁸, using a novel gel transfer technique, detected a 68K protein in T-negative cells. However, in these cases it would be difficult to define the basis of the reaction as the natural assumption would be that it represented a contaminating independent antibody activity rather than a cross-reaction.

The detection of a host cell protein sharing a common structure with a virally coded transforming gene product has already been described for the RNA tumour viruses and led to the hypothesis that the RNA virus has 'stolen' a host gene¹⁹. The present finding should not be interpreted in the same way, because, although the monoclonal antibody discussed here does recognize a structure common to a DNA virus oncogene and a normal host protein, the common structure may be very limited in extent. For example, most of the anti-T sera and the other anti-T monoclonal antibodies we have isolated do not recognize the 68K species and the T protein therefore has several unique antigenic determinants.

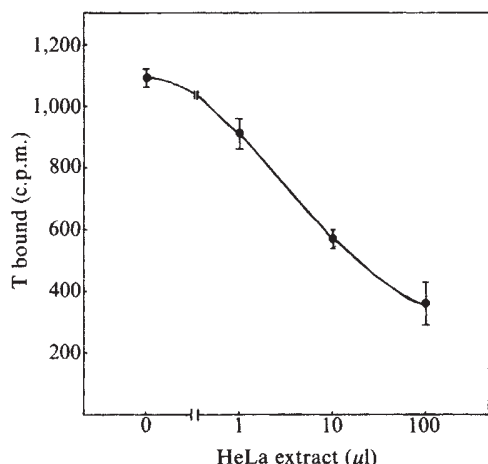


Fig. 4 Competition radioimmunoassay to detect the T-related protein. The HeLa cell extract was prepared by lysing 10^9 uninfected cells (grown in suspension culture) in 15 ml buffer (0.01 M phosphate, pH 7.0, 0.25% NP40 and 1 mM EDTA) on ice, centrifuging at 10,000g for 30 min and taking the supernatant fraction. Varying amounts of this cell extract were then mixed with a fixed amount of 35 S-labelled purified large T (isolated as in Fig. 1b) and sufficient 3C4 antibody added to precipitate 50% of the large T in the absence of any competition. The radioimmunoassay was then carried out as described¹³. Each point shows the mean and $\pm$ s.d. of a triplicate determination.

The extreme evolutionary conservation of the determinant recognized by 3C4 and the unusual nuclear localization of the host protein make it unlikely that the cross-reactivity is fortuitous, rather we would propose that the 68K protein and T share some common function. This function would involve an interaction with another host cell component and be highly conserved in evolution. In this model the interaction would be mediated through the region of T or of the 68K protein that form the 3C4 binding site; the SV40 T antigen thus mimics a structure on the host cell protein to usurp its function and thereby modify an aspect of the cells behaviour to suit the virus. Initial studies on the monoclonal antibody and the 68K protein support this: (1) 3C4 antibody is a potent inhibitor of the ATPase activity of the T-related protein made in Ad2/D2-infected HeLa cells (R. Tjian, personal communication). (2) The intensity of nuclear fluorescence seen with the antibody varies with the growth state of the cell, so that nuclei of quiescent rat embryo fibroblasts are barely positive whereas the same cells growing exponentially stain brightly. The 68K protein may be involved in the initiation of host DNA synthesis in much the same way as the T protein acts in the initiation of viral DNA synthesis. The two proteins would then share a common need to interact with the host cell DNA replication machine. Clearly the determination of the function of the 68K protein may aid our understanding of the role of T antigen both in lytic infection and in transformation.

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Mutants of avian myelocytomatosis virus with smaller gag gene-related proteins have an altered transforming ability

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Avian myelocytomatosis virus strain MC29 is a replication-defective¹ avian oncovirus which in newborn chickens causes myelocytomatosis and liver and kidney tumours^{2,3}. *In vitro* infection of bone marrow cells gives rise to colonies of transformed macrophage-like cells⁴, and cloned virus is also capable of transforming fibroblasts⁵. The genome of MC29 contains cellular sequences^{6–8} which are closely related to those in other defective leukaemia viruses with similar transforming spectra. Consequently, these cellular sequences have been postulated to represent a new oncogene which has been designated *mac*, for macrophage transformation^{4,9}. MC29-transformed cells contain a gag gene-related protein of a 110,000 molecular weight (MW) (p110)¹⁰, which by tryptic peptide analysis has been shown to be a fusion product comprised of gag gene-derived sequences and sequences which are presumed to be coded by the adjacent *mac* gene^{11,12}. These findings suggest that this protein may be implicated in transformation by MC29. We now describe three mutants of MC29 that synthesize smaller gag gene-related proteins. These mutants have an altered ability to transform bone marrow cells but not fibroblasts. This demonstrates for the first time a direct involvement of the p110 protein of MC29 in transformation.

The starting point of our studies was the observation that the Q10 cell line, a MC29-transformed quail fibroblast line producing MC29 plus MC29 associated helper virus (MCAV)¹⁰, on prolonged periods in culture synthesized smaller gag gene-related proteins in addition to p110. To determine whether we could isolate from this cell line virus mutants that would direct the synthesis of these smaller proteins, we infected fresh quail fibroblasts at a low multiplicity of infection and analysed single transformed clones for the synthesis of the gag-related proteins. Of 12 clones analysed, 6 produced a protein smaller than p110. Figure 1 shows an analysis by immune precipitation of non-producer clones transformed by three of the mutants (non-producer cells being cells transformed by MC29 but unable to release infectious virus due to the absence of helper virus). Cells transformed by MC29 virus clones 10A, 10C and 10H contained proteins of respective approximate MWs 100,000, 95,000 and 90,000. These proteins were shown to be related to the MC29 p110 protein by tryptic peptide analysis which revealed that they contained gag- and mac-specific peptides (data to be published elsewhere).

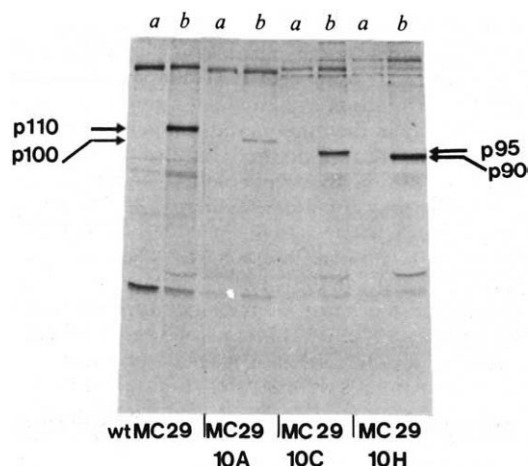


Fig. 1 Altered *gag* gene-related proteins synthesized in three clones of quail fibroblasts infected with MC29 virus derived from the MC29 Q10 line. Virus was collected from the supernatant fluids of the MC29 (MCAV) infected quail fibroblast line Q10 developed by Bister *et al.*¹⁰, and used to infect fresh quail embryo cells at various dilutions of the virus. Foci of transformed cells were isolated 10 days later from dishes (50 mm) containing less than 10 foci and grown up to approximately 2×10^7 cells. Clones were screened for p110-related proteins by radioimmunoprecipitation analysis. For this, 5×10^6 cells were labelled with ^{35}S -methionine for 60 min and *gag*-related proteins immunoprecipitated by anti-virus serum and analysed by SDS-polyacrylamide gel electrophoresis as detailed elsewhere¹⁰. Immunoprecipitates were made using either normal rabbit serum (a) or rabbit anti-RSV serum (b).

The mutant MC29 genomes were rescued from the non-producer cells by superinfection with the helper virus *td* B77 and the rescued viruses tested for their ability to transform fibroblasts and bone marrow cells in comparison with wild-type virus. Table 1 shows that although the three mutant viruses still transform fibroblasts with titres similar to wild-type virus they transform haematopoietic cells at an approximately 100-fold reduced efficiency. Wild-type MC29 transforms both cell types with roughly equal efficiency. In addition, the colonies of bone marrow cells transformed by the mutant viruses were smaller than the wild-type colonies and proved difficult to grow. This difference in efficiency of transformation of bone marrow cells can also be shown directly on transformation of chick embryo

Table 1 Transforming ability of the MC29 mutants assayed on chick embryo cells and chick bone marrow cells

Virus	Transforming activity			
	Chicken embryo cells		Chicken bone marrow cells	Ratio
	Fibroblasts foci (FFU ml ⁻¹)	Macrophage foci (FFU ml ⁻¹)	Macrophage colonies (CFU ml ⁻¹)	
wt MC29 (<i>td</i> B77)*	2.3×10^3	1.9×10^3	2×10^3	0.9
wt MC29 (RAV-2)	1.3×10^6	7.0×10^5	NT	0.5
MC29-10A (<i>td</i> B77)	5×10^4	3.2×10^2	6×10^2	0.009
MC29-10C (<i>td</i> B77)	1×10^6	NT	6×10^3	0.006
MC29-10H (<i>td</i> B77)	1×10^4	2×10^2	5×10^1	0.01

Non-producer clones of quail fibroblasts were superinfected with the avian leukosis virus *td* B77, passaged once and virus collected from the supernatant fluids 5–7 days later. FFU, focus-forming units. Titres were obtained following serial 10-fold dilutions of the virus stocks as described⁵. CFU, colony-forming units. The ratio was calculated by dividing the macrophage transforming titre, from CEC and bone marrow, by the fibroblast transforming titre. NT, not tested.

* The wild-type virus used was derived either from the Q8 fibroblast line¹⁰ superinfected with *td*B77 or from a MC29 (RAV-2)-producing macrophage cell line (H. Beug *et al.*, in preparation).

cells (CEC) that contain a mixture of cell types, including fibroblasts and macrophage-like cells (Table 1). Wild-type MC29 can transform both these cell types giving rise to small tight colonies of fibroblasts and large diffuse colonies of macrophage-like cells⁵. Figure 2 shows that CEC infected by two of these mutants, 10A and 10H, give rise exclusively to small tight fibroblast foci (Fig. 2a, b), whereas, as expected, wt MC29 gave rise to both types of focus (Fig. 2c). Cells newly infected with the mutant pseudotypes synthesized *gag*-related proteins corresponding in MW to those detected in the original isolates (unpublished observations).

The finding that a deletion in the p110 protein of MC29 leads to a large reduction in the macrophage transforming ability of the virus without affecting its fibroblast transforming potential could be taken to indicate that there are two separate gene products responsible for the transformation of different host cells. The fact that no messenger RNA other than that which codes for the MC29 p110 protein has been found in transformed cells (D. Stehelin *et al.*, personal communication) argues against this possibility. Another possibility is that there are domains in the molecule which are required for macrophage but not for fibroblast transformation. A third, not necessarily mutually exclusive alternative is that more transforming activity is required to transform bone marrow cells than is required to transform fibroblasts. The deletion observed in our mutants could have reduced the biological activity of the *gag*-related protein to a level which is insufficient to transform macrophages efficiently. The isolation of further DLV mutants altered in their ability to transform fibroblasts but still able to transform bone marrow cells would be useful in resolving this question. Interestingly, a mutant of avian erythroblastosis virus has been isolated which has also lost the ability to transform bone marrow cells but is still able to transform fibroblasts¹³. This mutant also has a deletion in the *gag* gene-related protein (p75) of this particular virus¹⁴.

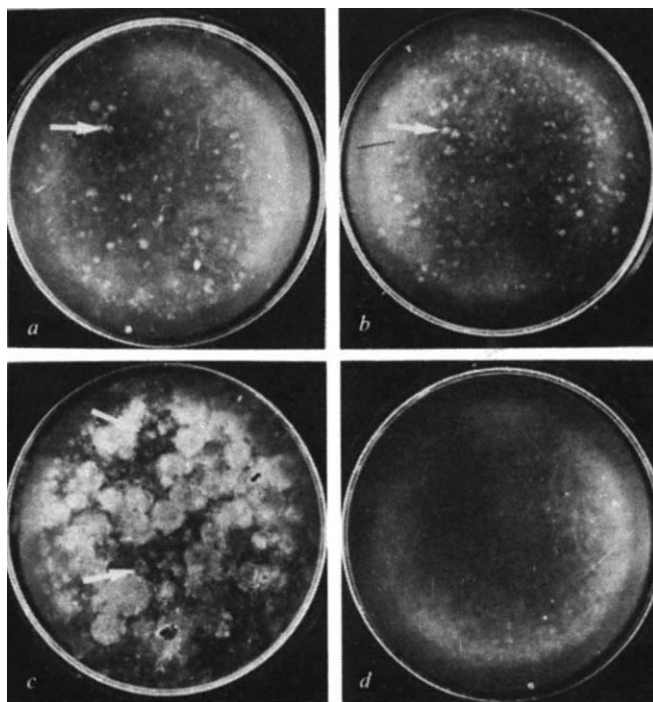


Fig. 2 Foci of chick embryo cells infected with mutants of MC29. The focus assay was prepared as described earlier⁵ and pictures taken 10 days after infection. a, Foci induced by *td* 10A; b, by *td* 10H; and c, by wt MC29. All dishes were inoculated with 0.2 ml of virus containing approximately 200 FFU. d, Uninfected control. The foci in a and b as well as the small foci in c (indicated by the arrows) represent fibroblast foci. The large plaque-like foci seen only in c represent foci derived from transformed macrophages.

Studies are under way to locate the alterations in the proteins of the MC29 mutants by tryptic peptide mapping techniques and oligonucleotide mapping. Preliminary results indicate that the mutant proteins have lost several *mac*-specific peptides and have gained a *gag*-specific peptide and this can be correlated with a deletion(s) in the *mac* sequences in the genomic RNA (G.R., K. Bister, P. H. Duesberg and M.J.H., preliminary observations). This could indicate that our MC29 mutants arose by crossing-over between helper leukemia virus and the MC29 genome.

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X-chromosome inactivation in extra-embryonic membranes of diploid parthenogenetic mouse embryos demonstrated by differential staining

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In somatic cells of female mammals one of the two X chromosomes is genetically inactive and heterochromatic, resulting in dosage compensation for X-linked genes¹⁻³. In marsupials the paternally derived X chromosome is preferentially inactivated. In eutherian mammals, although either X chromosome can be inactivated at random in somatic cells, preferential inactivation of the paternally derived X chromosome has been demonstrated cytologically in mouse and rat yolk sac^{4,5} and mouse chorion⁶ and biochemically in mouse yolk sac⁷, chorionic ectoderm⁸ and trophoblast. In mouse yolk sac the non-random element has been shown both biochemically⁷ and cytologically⁹ to be confined to the endoderm layer in which there is almost total paternal X-chromosome inactivation. We have therefore looked at X-chromosome activity in the separated yolk sac layers of diploid parthenogenetic mouse embryos in which both X chromosomes are maternally derived. Kaufman *et al.*¹⁰ have demonstrated X inactivation in somatic cells of diploid parthenogenetic embryos, and we have used a modification of Kanda's method¹¹, which renders the presumptive inactive X dark staining, to reveal an inactive X chromosome in both endoderm and mesoderm layers of separated yolk sacs from parthenogenones. Thus even in tissues in which there is normally total non-random paternal X inactivation, in the absence of a paternally derived X chromosome a maternally derived X can be inactivated.

Kanda identified the inactive X chromosome at metaphase in female mice¹¹ and other rodents¹² by treating bone marrow cells with hot hypotonic potassium chloride solution before fixation. The inactive X chromosome then stained much more darkly with Giemsa dyes than the other chromosomes in the cell. The modification of the Kanda method for use with embryonic material involved placing the whole embryo and/or embryonic membranes for 90 min in culture medium 199 (Flow Laboratories) to which colchicine had been added ($4 \mu\text{g ml}^{-1}$ final concentration) to accumulate metaphases. The embryos were then placed individually in Petri dishes containing 0.5% (w/v) potassium chloride solution at room temperature and incubated in a water bath at 50 °C for 15 min, followed by fixation in freshly made 3:1 absolute ethanol/glacial acetic acid solution. After fixation the embryos were isolated, touched lightly to filter paper to remove excess fixative and disaggregated in 0.5 ml of 60% acetic acid for 5 min. Chromosome spreads were made by allowing small drops of the acetic acid mixture to evaporate on slides placed on a 40 °C hot plate. The preparations were stained in 2% Giemsa buffered at pH 6.8 for 20 min.

A dark-staining X chromosome was seen in more than 80% of metaphase cells from female embryos at $5\frac{1}{2}$, $6\frac{1}{2}$, $7\frac{1}{2}$ and $13\frac{1}{2}$ days post-coitum (p.c.) (Table 1 and Fig. 1a, b). Strong evidence that the dark staining chromosome is the inactive X chromosome is that it is seen in such a high proportion of XX female cells, but not in XO female cells nor XY male cells. A dark staining X chromosome was also present in more than 75% of cells from a bone marrow preparation of an adult XX male mouse carrying *Sxr*, the autosomal gene for sex reversal¹³.

Five experimental diploid parthenogenetic embryos were produced by the method of Kaufman *et al.*¹⁴ by suppression of second polar body formation, and dissected out of their decidua at a stage equivalent to $9\frac{1}{2}$ - $10\frac{1}{2}$ -day gestation (10-25 somites present). Their yolk sacs were removed, washed in medium 199 and placed in medium 199 with colchicine ($4 \mu\text{g ml}^{-1}$ final concentration) for 3 h at room temperature to accumulate metaphases. The yolk sacs were then separated into their endodermal and mesodermal components by partial digestion for 3 h in a mixture of 2.5% pancreatin and 0.5% trypsin (w/v) made up in Ca^{2+} and Mg^{2+} -free Tyrode solution at 4 °C¹⁵, followed by dissection with watchmaker's forceps. The cleanly separated cell layers were then individually treated by the modified Kanda method, as described above. Chromosome spreads from the two separated yolk sac components from normal fertilized zygotes at the same developmental stage were also obtained. The slides were all stained in 2% Giemsa at pH 6.8 for 20 min, randomized and coded, and cells at metaphase were scored for the presence or absence of a dark staining X chromosome (Table 2). A dark staining X

Table 1 Staining of metaphase cells from female embryos

Age of embryos (days p.c.)	No. of female embryos	Total metaphases	Metaphases with a dark X chromosome	Per cent with a dark X chromosome
$5\frac{1}{2}$	8	221	192	86.88
$6\frac{1}{2}$	13	1,188	1,019	85.77
$7\frac{1}{2}$	6	545	455	83.49
$13\frac{1}{2}$	8	598	495	82.78

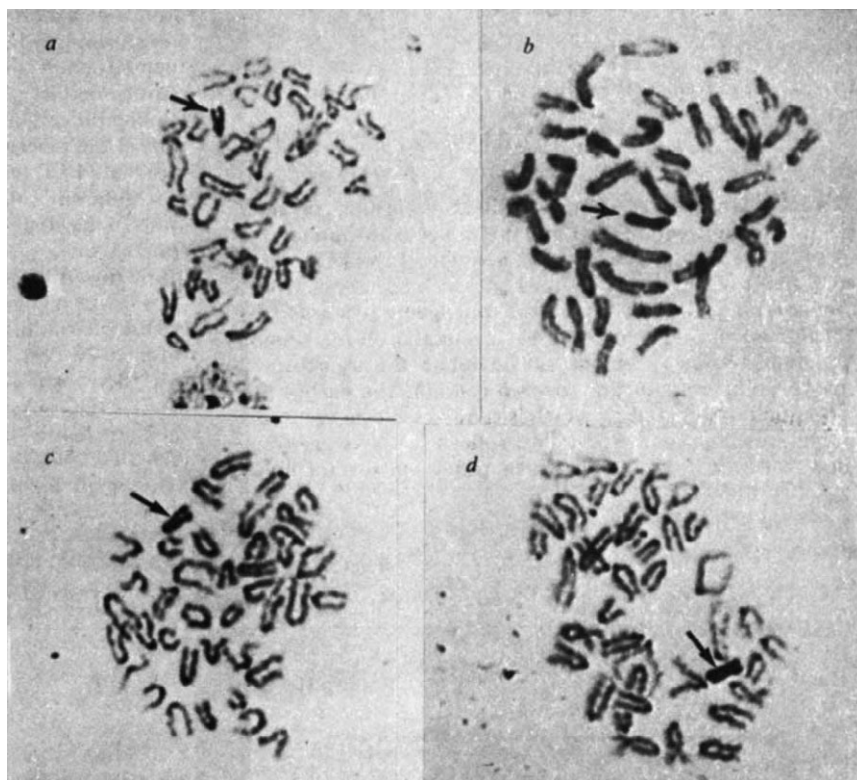
Virgin female mice of the 3H1 strain (F_1 hybrids between two inbred strains, C3H/HeH and 101/H) were injected with 5 IU pregnant mare's serum gonadotropin (PMS) followed 44 h later by an injection of 5 IU human chorionic gonadotropin (HCG) to synchronize ovulation, and were mated to 3H1 males. Females were checked for vaginal plugs early the next morning, and the day of the plug was designated day $\frac{1}{2}$. Pregnant females were killed by cervical dislocation at various stages of pregnancy, and the embryo was dissected out under a dissecting microscope. Whenever possible Reichert's membrane was removed to ensure that there was no contamination by maternal cells. The $13\frac{1}{2}$ -day embryos were sexed by dissecting out the gonad, which has visibly differentiated by this stage, and the earlier embryos were sexed cytologically.

Table 2 Staining of metaphases from yolk sacs

Tissue	No. embryos		No. metaphases scored		No. metaphases with a dark X chromosome	
	Parthenogenones	Controls	Parthenogenones	Controls	Parthenogenones (%)	Controls (%)
Yolk sac endoderm	5	14	94	914	76(80.85)	742(81.18)
Yolk sac mesoderm	5	14	328	675	255(77.74)	558(82.67)

This table shows the number of cells at metaphase from parthenogenetic separated yolk sac endoderm and mesoderm exhibiting a dark-staining X chromosome after treatment with the modified Kanda method, compared with controls of fertilized material which have received the same treatment.

Fig. 1 Metaphase cells showing an inactive dark-staining X chromosome after hypotonic treatment at 50 °C. *a*, Metaphase from a normal 5½-day p.c. embryo; *b*, metaphase from a normal 7½-day p.c. embryo; *c*, metaphase from the endoderm layer of diploid parthenogenetic yolk sac; and *d*, metaphase from the mesoderm layer of diploid parthenogenetic yolk sac.



chromosome was seen in 76 out of 94 metaphases in parthenogenetic yolk sac endoderm (Fig. 1c) and in 255 out of 328 metaphases scored from parthenogenetic yolk sac mesoderm (Fig. 1d). We therefore conclude that X-chromosome inactivation has taken place in both endodermally and mesodermally-derived layers of yolk sac from diploid parthenogenones.

Cooper¹⁶ has suggested that passage of the X chromosome through male gametogenesis or fertilization led to imprinting of the paternal X chromosome and was an important factor in paternal X inactivation in marsupials. It has been further suggested that imprinting also occurs in eutherian mammals¹⁷ and results in paternal X inactivation in those tissues of the embryo that differentiate early, such as yolk sac endoderm which is derived from the primitive endoderm¹⁸, but that cells of the embryo proper escape from imprinting before the time of X-chromosome differentiation so that in them X inactivation is random^{19,20}. Our results show that even in a tissue such as yolk sac endoderm in which primary non-random inactivation of the paternal X is thought to occur, a maternal X chromosome can be inactivated in the absence of a paternal one. In our parthenogenetic material neither X chromosome could have been imprinted, either by passage through male gametogenesis, fertilization or re-entry of the polar body, and so we conclude that imprinting cannot be essential to the mechanism of X inactivation in the tissues in which non-random paternal X inactivation normally occurs. One cannot, however, assume that absence of an imprinted X chromosome has not affected X inactivation in any way; for example, is the onset of X inactivation

in these tissues delayed? Homozygous diploid uniparental mice, produced by the removal of one or other pronucleus after fertilization, followed by diploidization in cytochalasin B, are viable²¹, which suggests that X inactivation in all tissues has occurred normally. However, as no live-born mammalian parthenogenones have been produced so far, one cannot rule out the possibility that some abnormality of X inactivation is involved in their premature death.

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Structure of two adenovirus type 12 transforming polypeptides and their evolutionary implications

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The human adenoviruses are classified according to their nucleotide sequence homology and their oncogenic potential in rodents^{1,2}. The left-hand end of the genome of the adenovirus types 5 (ad5), 7 (ad7) and 12 (ad12), which are respectively, non-oncogenic (subgroup C), weakly oncogenic (subgroup B) and highly oncogenic (subgroup A)³⁻⁵, contains all the genetic information needed to induce and maintain the transformed phenotype. This part of the genome contains the early transcription unit designated E₁ which is subdivided into two transcription units E₁A and E₁B⁶. Two spliced mRNAs are transcribed from the E₁A region which codes for several phos-

phorylated polypeptides. These polypeptides play a key role by controlling the expression of the other early transcription units^{7,8}. The major role of region E₁A in adenovirus cell transformation might be to activate the true transforming genes of the region E₁B. An additional role probably consists of the activation of some cellular genes as a restriction fragment containing this region can immortalize rodent cells *in vitro*. An important question is why some adenoviruses are oncogenic and others are not. We report here differences in the structures of the E₁A polypeptides from ad7 and ad12, compared to ad5, which may partially account for their differing oncogenicity.

To answer this question, we have cloned in *Escherichia coli* double-stranded complementary DNA (cDNA) copies of the transforming mRNAs from region E₁A of ad12 and compared their structures and their translation products with those of the corresponding mRNAs of ad5 (ref. 9) and ad7 (ref. 10). The cloning procedure and the determination of the physical structure of the cDNA were as described in Fig. 1. The two clones designated 11 and 44 contain the longest inserts and describe two different spliced mRNAs of the region E₁A. Structural study of the cloned cDNAs at the nucleotide level shows that the splicing events of the mRNAs result in the deletion of a markedly AT-rich region, between nucleotides 1,069 and 1,144, which contains many stop codons¹¹ (Fig. 2). In clone 44 position 976 (donor site D₁) is spliced to position 1,144 (acceptor site A), and in clone 11 position 1,069 (donor site D₂) is spliced to position 1,144 (Fig. 1). The sequence of the donor site D₁ (UU↓GUAAGU) is unexpected as a pyrimidine precedes the dinucleotide GU. The sequence of the other donor site D₂ (CA↓GUAAGU) is very similar to the prototype sequence for a donor site described by Seif *et al.*¹².

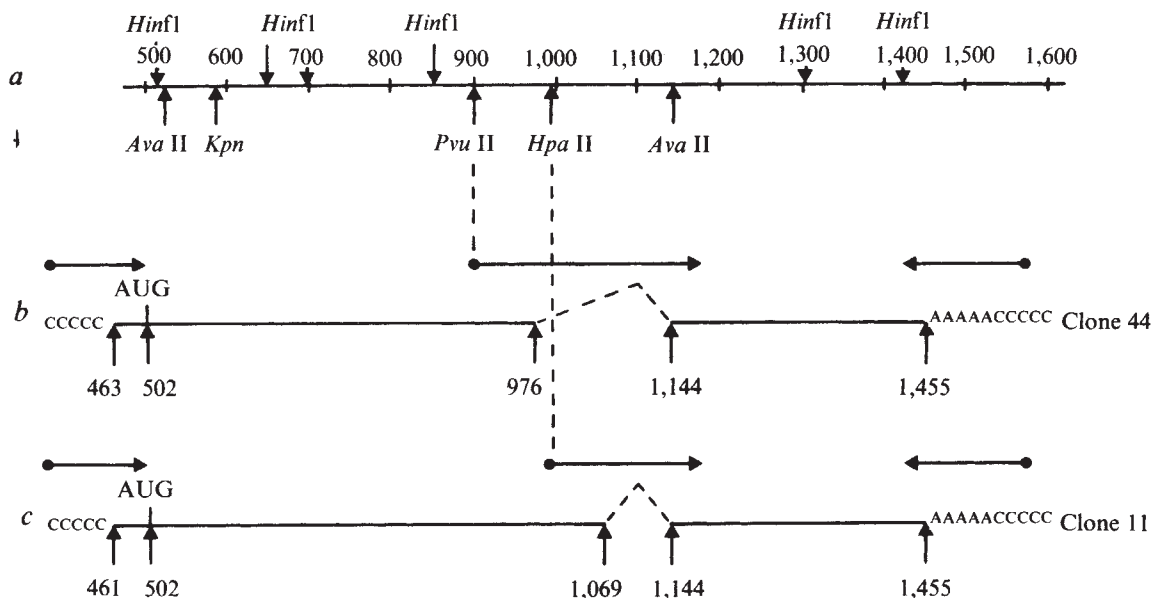


Fig. 1 Structure of the two mRNAs of the E₁A region from ad12 genome. cDNA copies of RNA from ad12-infected KB cells, 8-h post-infection and grown in the presence of cycloheximide were synthesized as described elsewhere¹⁴ except that RNA was not poly(A) selected. cDNA was inserted into the PstI restriction site of plasmid pBR322 after dG-dC tailing with the terminal transferase⁹. The recombinant plasmids were propagated in the *E. coli* DP 50 bacterial strain. Bacterial clones containing ad12-specific sequences of the transforming region were identified by *in situ* hybridization using the EcoRI-C restriction fragment (0–16%) as a probe; 30 clones gave a positive hybridization signal. Subsequent hybridization using ³²P-labelled poly(dT) as a probe was then performed to detect clones containing a poly(A) tract. Six clones gave a positive signal. Plasmid was extracted from the 30 clones and analysed by restriction endonuclease digestion. The size of the insert was determined by sizing of the PstI restriction fragment and the plasmids containing the longest cDNAs as well as a poly(A) tail were selected for further analysis. Plasmid, ³²P-labelled *in vitro* by nick translation, hybridized only to the HindIII-G restriction fragment (0–6.8% of the genome) and therefore must contain cDNA sequences corresponding to mRNA of region E₁A. *a* Represents the physical map of a part of the left-hand end of the ad12 genome. *b* Represents the cDNA structure from clone 44. It is 900 base pairs long and possesses the AvaII restriction site at nucleotide 525, but not the PstI restriction site at position 1,143 suggesting it is located in the intron. The five HindIII restriction sites are present and the HindIII DNA fragment located between positions 853 and 1,307 is about 150 base pairs shorter than the corresponding genomic DNA fragment suggesting that this fragment contains the splice junction. *c* Represents the cDNA structure from clone 11. It is 1,000 base pairs long and possesses the same restriction sites as clone 44. However, the HindIII DNA fragment located between positions 853 and 1,307 is 75 base pairs shorter than the homologous genomic restriction fragment, suggesting that this fragment contains different splice junctions. The 5' end, the polyadenylation site and the splice points were located by the nucleotide sequence determination of the appropriate region. Sequence analysis was carried out according to the Maxam and Gilbert procedure¹⁵. The horizontal arrows show the strategy adopted for our sequencing approach. Due to the presence of tandem repeats at the splice points (GU in both mRNAs) it is not possible to pinpoint precisely the positions where splicing has taken place. However, by following the GU/AG rule, tentative location for the splice points can be established and are indicated by vertical arrows. Following this assumption, nucleotide 976 is connected to nucleotide 1,144 in the mRNA corresponding to the clone 44 (*b*) and nucleotide 1,069 is connected to nucleotide 1,144 in the mRNA corresponding to clone 11 (*c*).

502
GAG TTT TCT CTG CCA GCT CAT TTT CAC GGC GCC ATT ATG AGA ACT GAA ATG ACT CCC TTG
Met Arg Thr Glu Met Thr Pro Leu

GTC CTG TCG TAT CAG GAA GCT GAC GAC ATA TTG GAG CAT TTG GTG GAC AAC TTT TTT AAC
Val Leu Ser Tyr Gln Glu Ala Asp Asp Ile Leu Glu His Leu Val Asp Asn Phe Phe Asn

GAG GTA CCC AGT GAT GAT GAT CTT TAT CTT CCG TCT CTT TAC GAA CTG TAT GAT CTT GAT
Glu Val Pro Ser Asp Asp Asp Leu Tyr Val Pro Ser Leu Tyr Glu Leu Tyr Asp Leu Asp

GTG GAG TCT GCC GGT GAA GAT AAT AAT GAA CAG GCG GTG AAT GAG TTT TTT CCC GAA TCG
Val Glu Ser Ala Gly Glu Asp Asn Asn Glu Gln Ala Val Asn Glu Phe Phe Pro Glu Ser

CTT ATT TTA GCT GCC AGT GAG GGG TTG TTT TTA CCG GAG CCT CCT GTA CTT TCT CCT GTC
Leu Ile Leu Ala Ala Ser Glu Gly Leu Phe Leu Pro Glu Pro Pro Val Leu Ser Pro Val

TGT GAG CCT ATT GGG GGC GAA TGT ATG CCA GAA CTG CAC CCT GAA GAT ATG GAT TTA TTG
Cys Glu Pro Ile Gly Glu Cys Met Glu His Pro Glu Asp Met Asp Leu Leu

TGC TAC GAG ATG GGC TTT CCC TGT AGC GAT TCG GAA GAC GAG CAA GAC GAG AAC GGA ATG
Cys Tyr Glu Met Gly Phe Pro Cys Ser Asp Ser Glu Gln Asp Glu Asn Gly Met

GCG CAT GTT TCT GCA TCC GCA GCT GCT GCT OCC OCT GAT AGG GAA COT GAG GAG TTT CAG
Ala His Val Ser Ala Ser Ala Ala Ala Ala Ala Asp Arg Glu Arg Glu Glu Phe Gln

TTA GAC CAT CCA GAG TTG CCC GGA CAC ATT TGT AAG TCC TGT GAG CAC CAC CGG AAT AGT
Leu Asp His Pro Glu Leu Pro Gly His Asn Cys Lys Ser Cys Glu His His Arg Asn Ser

ACT GGA AAT ACT GAC TTA ATG TGC TCT TTG TGC TAT CTG CGA GCC TAC AAC ATG TTC ATT
Thr Gly Asn Thr Asp Leu Met Cys Ser Leu Cys Tyr Leu Arg Ala Tyr Asn Met Phe Ile

TAC A GTAAGTGTCTATGGAGGTGGAGGTGATTTTTTTCTTAAGCAGTGAAAAATATTTTGTGTTTAG
Tyr Ser

GT CCT GTT TCC GAT AAT GAG CCT GAA CCT AAT AGC ACT TTG GAT GGC GAT GAG CGA CCC
Pro Val Ser Asp Asn Glu Pro Glu Pro Asn Ser Thr Leu Asp Gly Asp Glu Arg Pro

TCA CCC CCG AAA CTA GGA AGT GCG GTT CCA GAA GGA GTA ATA AAA CCT GTG CCT CAG CGG
Ser Pro Pro Lys Leu Gly Ser Ala Val Pro Glu Gly Val Ile Lys Pro Val Pro Gln Arg

GTG ACT GGG AGG COT AGA TGT GCT GTG GAA AGC ATT TTG GAT TTG ATT CAA GAG GAA GAA
Val Thr Gly Arg Arg Arg Cys Ala Val Glu Ser Ile Leu Asp Leu Ile Gln Glu Glu Glu

AGA GAA CAA ACA GTG CCT GTT GAT CTG TCA GTG AAA CGC CCT AGA TGT AAT TAA TGG ACT
Arg Glu Gln Thr Val Pro Val Asp Leu Ser Val Lys Arg Pro Arg Cys Asn

TTG AGC ACC TGG GCA ATA AAA TAG GGG TAA TOT GGT TTT TGT GAG TCA TGT ATA ATA AAA

GTG GTC TCG GTT GAA GTG TCT TGT TAA TGT TTG TTT

Fig. 2 The proposed primary structure for the two mRNAs and for the corresponding polypeptides of the ad12 E₁A region. These structures are deduced from our analysis of the cDNA clones together with the nucleotide sequence for the left-hand end of ad12 DNA. The sequence which is absent in the shortest mRNA is underlined. The hexanucleotide AATAAA present close to the poly (A) junction of eukaryotic mRNA¹⁶ is indicated by a box. Nucleotides which become connected after splicing are shown in boxes (see also Fig. 3). The nucleotide sequence is indexed according to the nomenclature of Sugisaki *et al.*¹⁷.

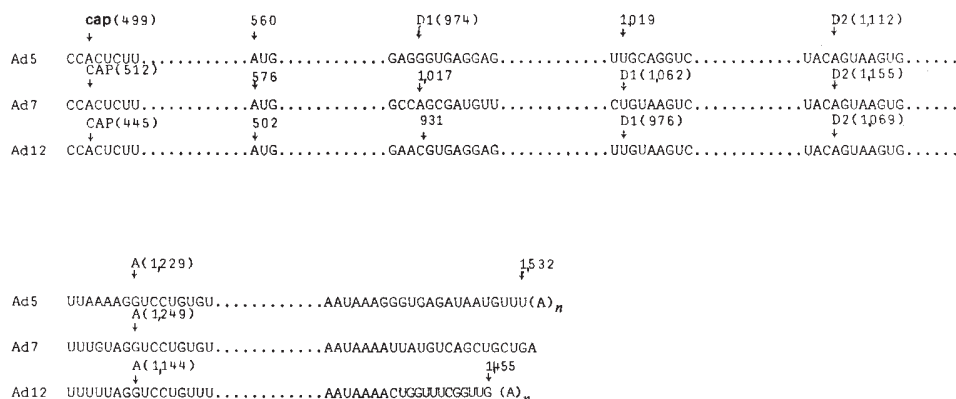


Fig. 3 Comparison of the homologous regions for donor and acceptor sites of the mRNAs from the E₁A early region of ad5, ad7 and ad12. The nucleotide sequences of the ad5 capping site are from ref. 18. The nucleotide sequences of the ad5 splicing and poly(A) sites are from ref. 9. The nucleotide sequences of the ad7 capping and splicing sites are deduced from comparing the homologous nucleotide sequences of ad5 (ref. 13), ad7 (ref. 10) and ad12 (ref. 11) genomes. The nucleotide A, position 1,017 of ad7 from the region homologous to the ad5 D₁ site, cannot be used as a splice donor site as it is followed by the dinucleotide GC. On the contrary the sequence UGUAAGU of the ad12 D₁ site (U: donor nucleotide) is found around the ad7 nucleotide U, position 1,062. The donor site D₂ and the acceptor site A of ad7 have been localized respectively at positions 1,155 and 1,249 by a similar method.

Sequence analysis from the *Pst*I cleavage sites of both clones 11 and 44 allowed us to describe the boundaries of the mRNAs. Sequencing gels revealed the same poly(A) addition sites for both mRNAs. However, it was not possible to predict exactly where the poly(A) addition had occurred because two As follow the G residue at position 1,455 in the DNA sequence¹¹ (Fig. 2) as is frequently the case for eukaryotic mRNAs. The poly(A) addition site is located 14–16 nucleotides after the typical hexanucleotide sequence AAUAAA. Note that two other hexanucleotide sequences AAUAAA are encountered at positions 1,397 and 1,241. However, no poly(A) addition seems to occur downstream of these positions, thus indicating that the hexanucleotide sequence alone may not be a sufficient signal for polyadenylation. Sequence analysis performed at the 5' ends of both cDNAs located the 5' boundaries at nucleotides 461 and 463, respectively, for clones 11 and 44, that is about 40 nucleotides upstream from the AUG presumed to be the initiation codon (Fig. 1). However, these positions probably do not represent the true 5' ends of the mRNAs because a few nucleotides are usually lost at the extremities during mRNA cloning. The cap site is probably located at position 445 as the sequence at this position is identical to the sequence at the cap site in region E₁A of ad5 (Fig. 3).

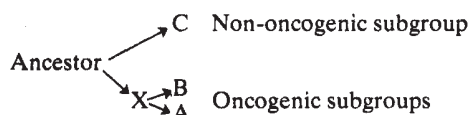
A comparison of the sequences for donor and acceptor sites of ad5, ad7 and ad12 is very informative (Fig. 3). The results from our cloning experiments show that the donor sites D₂ and the acceptor sites A are located in homologous sequences in both serotypes 5 and 12 whereas the donor sites D₁ are not. Thus donor site D₁ is displaced 45 nucleotides further along the serotype 12 sequence as compared to the homologous sequence of serotype 5. Although the splices for the E₁A mRNAs from ad7 have not yet been established by cloning experiments, they can be predicted by comparing the DNA sequences of the three serotypes 7, 12 and 5 (refs 10, 11 and 13, respectively) around the presumed splice points. Thus, the only alternatives are the positions 1,062 and 1,155 for donor sites D₁ and D₂ and nucleotide 1,249 for the acceptor site. The donor site D₁ would be located in homologous sequences in both serotypes 7 and 12 and the dinucleotide GU would in both cases be preceded by a U residue. The donor site D₂ and the acceptor site A would be located in homologous sequences in the three serotypes.

The established DNA sequence together with the positions of splice points allow us to predict the primary sequences of the polypeptides which are encoded by each of the ad12 E₁A spliced mRNAs (Fig. 2)¹¹ and to compare them with those of the two other serotypes 5 (refs 13, 9) and 7 (ref. 10) to find common features (Fig. 4). In the three serotypes both mRNAs are translated before and after the splice. Thus for each serotype the two E₁A polypeptides have identical amino- and carboxy-

Fig. 4 Diagram showing the homologous amino acids occurring in the longest E₁A polypeptides among the three adenovirus types 5, 7 and 12. Black areas (■) represent homologies and colourless areas non-homologies. Ile, Leu and Val, Ser and Thr, Asn and Gln, Asp and Glu, were respectively considered as similar amino acids. Gaps are left whenever necessary to maximize homology. The vertical arrow indicates the position where the intron is found in the genome. The black areas show the good conservation of the amino acid sequences of the three polypeptides. However, a deletion corresponding to about 40 amino acids and an insertion corresponding to about 20 amino acids are present in both ad7 and ad12 compared to ad5. The left part of the figure corresponds to the N-terminal end of the polypeptides. Scale bar denotes a peptide 10 amino acids long.



terminal ends, their only difference being an internal deletion due to different positions of the donor sites for splicing. A comparison of the amino acid sequences shows an important deletion located in the C-terminal part of the polypeptides, and an insertion and another deletion in homologous regions of ad7 and ad12 compared to ad5. However, several identical amino acid stretches are encountered along the three polypeptide sequences showing the existence of highly conserved regions. This suggests a common ancestor for these adenoviruses. The different locations of the splice sites and the differences observed in the primary structures of the polypeptides also suggest that subgroups A and B had some evolutionary steps in common. A scheme for the evolutionary path might be:



The different primary structures observed for the ad7 and ad12 E₁A polypeptides compared to ad5 might explain in part the oncogenic properties of these serotypes. Nevertheless, a similar comparison of primary structures of ad12 E₁B polypeptides with those of ad7 and ad5 is necessary. Among the 10,000 clones so far analysed, not one was found to contain sequences from region E₁B. A hybridization experiment between single-stranded ³²P-DNA copies of ad12 mRNA and

ad12 HindIII restriction fragments, showed no signal for the HindIII-I restriction fragment (6.8–10.9% of the genome). This result suggests that the region E₁B is not transcribed early during the lytic cycle. Therefore there may be a fundamental difference in the expression of region E₁B in adenovirus subgroups A and C. Further studies of the protein products from the E₁B region are clearly required to resolve this issue and we are currently extending our analysis to the region E₁B.

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Phorbol ester-induced differentiation of chronic lymphocytic leukaemia cells

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The croton oil-derived tumour-promoting agent 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) exerts pleiotropic effects on the differentiation and proliferation of both normal and malignant animal and human cells *in vitro*. TPA is mitogenic in nanomolar concentrations to chicken embryo fibroblasts¹ and human T lymphocytes² and inhibits the terminal differentiation of various committed embryonic cells^{3,4} and mouse Friend erythroleukaemia⁵ or myeloid leukaemia⁶ cells. TPA induces a terminal cell differentiation in some murine⁷ and human^{7–11} myeloid leukaemia and histiocytic lymphoma cells¹². We report here the effect of TPA on chronic lymphocytic leukaemia (CLL) biopsy cells *in vitro*. In four out of five CLL patients studied, TPA induced the appearance of 90–100% lymphoblastoid and plasmacytoid cells after 4 days of culture. Under the influence of TPA, 86–97% of the cells expressed with time increasing amounts of intracytoplasmic immunoglobulin (C-Ig) of the same phenotype as that detected on the surface (S-Ig) of fresh, non-induced CLL cells. A parallel decrease in both monoclonal S-Ig density and DNA synthesis of the CLL cells was observed. Electron microscopic studies showed a maturation towards plasma cells. We therefore conclude that TPA is capable of inducing differentiation of CLL cells *in vitro*.

Three patients with CLL (AK, EJ and MW) and one patient (EB) with prolymphocytic leukaemia (a clinical variant of CLL¹³) were selected for this study on the basis of high blood levels ($>10^5 \text{ mm}^{-3}$) of monoclonal B lymphocytes. Blood mononuclear cells were separated by density-gradient centrifugation¹⁴ and analysed for relative numbers of E rosetting¹⁵ (on average 8%) and S-Ig-positive cells (on average 91% stained cells using polyvalent fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human Ig F(ab')₂ fragment, Kallestad). The S-Ig isotypes of the cells were determined using fluorescent F(ab')₂ antisera specific for human heavy (μ , δ , γ , α , ϵ) and light (κ , λ) chains (Kallestad; anti- ϵ serum produced by Behring).

In preliminary experiments, the cells were plated and cultured with different concentrations of TPA. Figure 1 shows that the maximal frequency of C-Ig-positive cells ($>90\%$ at day 3) was found with a TPA concentration of $1.6 \times 10^{-7} \text{ M}$. This concentration was used in later experiments.

Figures 2, 3 and 4 demonstrate the morphological features of CLL cells exposed to TPA. Within 4–12 h after plating, most of the cells became larger and acquired variable shapes. About 70% formed free-floating or surface-adherent clusters which increased in size with time (Fig. 3b). MGG-stained cytocentrifuge preparations at day 1–2 revealed a rapid enlargement of the cytoplasm together with an increasing basophilia and the appearance of a prominent nucleolus (lymphoblastoid cells). At day 3–4, cells with a large basophilic and frequently vacuolized cytoplasm and an eccentrically located nucleus (plasmacytoid cells) dominated the cultures together with the lymphoblastoid cells; only ~5% small lymphocytes were observed (Figs 2, 3c). Morphologically mature plasma cells were, however, only rarely seen. The viability remained $>90\%$ in both the TPA-treated and control cultures as judged by Trypan blue exclusion. The

Table 1 Effect of TPA on the expression and density of monoclonal surface and cytoplasmic immunoglobulin (S- and C-Ig) in CLL cells

Day of culture	Treatment of cells	Patient															
		EB (μ, λ)				AK (μ, δ, λ)				EJ (μ, λ)				MW (μ, δ, λ)			
		Staining for μ chains		S-Ig		Staining for μ chains		S-Ig		Staining for μ chains		S-Ig		Staining for μ chains		S-Ig	
		%	I	%	I	%	I	%	I	%	I	%	I	%	I	%	I
0	—	93	13	5	5	95	10	7	7	96	18	9	10	77	12	1	3
1	Control	82	13	6	6	73	6	4	7	85	16	4	6	ND	ND	ND	ND
	TPA	20	7	28	8	10	7	62	8	61	11	58	16	ND	ND	ND	ND
2	Control	47	6	2	2	75	6	6	9	67	9	24	7	ND	ND	ND	ND
	TPA	10	6	72	16	8	7	78	19	17	4	87	18	ND	ND	ND	ND
3	Control	52	6	14	7	ND	ND	8	7	71	7	16	11	83	10	4	4
	TPA	11	6	80	46	ND	ND	96	30	8	4	90	15	19	4	96	13
4	Control	42	6	8	8	74	8	11	8	58	5	11	4	ND	ND	ND	ND
	TPA	4	6	86	32	4	6	97	38	10	3	88	18	ND	ND	ND	ND

The S-Ig isotype of the CLL cells is given in brackets for each of the four patients (EB, AK, EJ, MW). Fresh biopsy cells (day 0) or cells cultured (day 1–4) in the absence (control) or presence of TPA (1.6×10^{-7} M) were stained for surface and cytoplasmic μ chains using FITC-coupled goat anti-human μ serum (F(ab')₂ fragment, Kallestad at dilutions 1:3 and 1:10, respectively). The proportion of stained cells (%; 250 cells scored) and mean intensity (*I*, relative units; 20–30 cells measured per sample) of cellular fluorescence were measured using a Zeiss surface fluorescence cytophotometer (UV light source HBO 100 W, excitation filter BG12, barrier filter 50; objectives Neofluar Ph2 40 $\times$ and Ph3 100 $\times$ for C-Ig and S-Ig, respectively). ND, not determined.

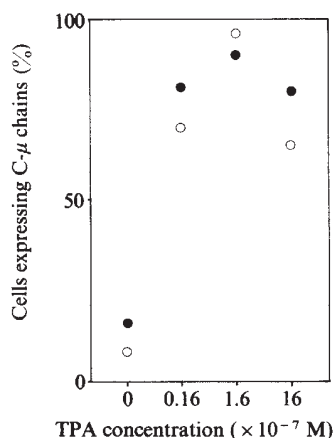


Fig. 1 Effect of different concentrations of TPA on the relative number of CLL cells expressing monoclonal cytoplasmic immunoglobulin. ○, Patient AK (S-Ig isotype μ, δ, λ); ●, patient EJ (μ, λ). CLL cells (10^6 ml^{-1}) were cultured on plastic Petri dishes in Ham's F-10 medium supplemented with 10% fetal calf serum, antibiotics and TPA (Sigma, stock solution 1 mg ml^{-1} dissolved in 100% ethanol) at indicated final dilutions. Cultures were collected on day 3. The cells were washed, cytocentrifuged on to microscope slides, fixed in ice-cold methanol:acetone (1:1) and stained for intracytoplasmic μ (C- μ) chains using specific 1:10 diluted FITC-conjugated rabbit anti-human μ serum (F(ab')₂ fragment, Kallestad). Fluorescent cells were quantified using the microscope equipment described in Table 1 legend.

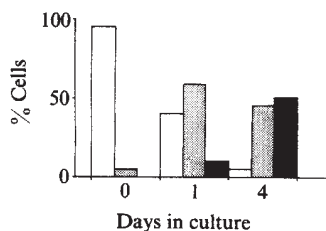


Fig. 2 TPA-induced morphological differentiation of CLL cells. Relative distribution (means for four CLL patients) of small lymphocytes (open bars), lymphoblastoid cells (shaded bars) and plasmacytoid cells (closed bars) at different times of culture. Cells were cultured in the continuous presence of 1.6×10^{-7} M TPA, collected on days 1 and 4, cytocentrifuged on to microscope slides and stained with May-Grünwald-Giemsa (MGG). Differential counts were calculated from 250 scored cells. Day 0 = untreated biopsy cells.

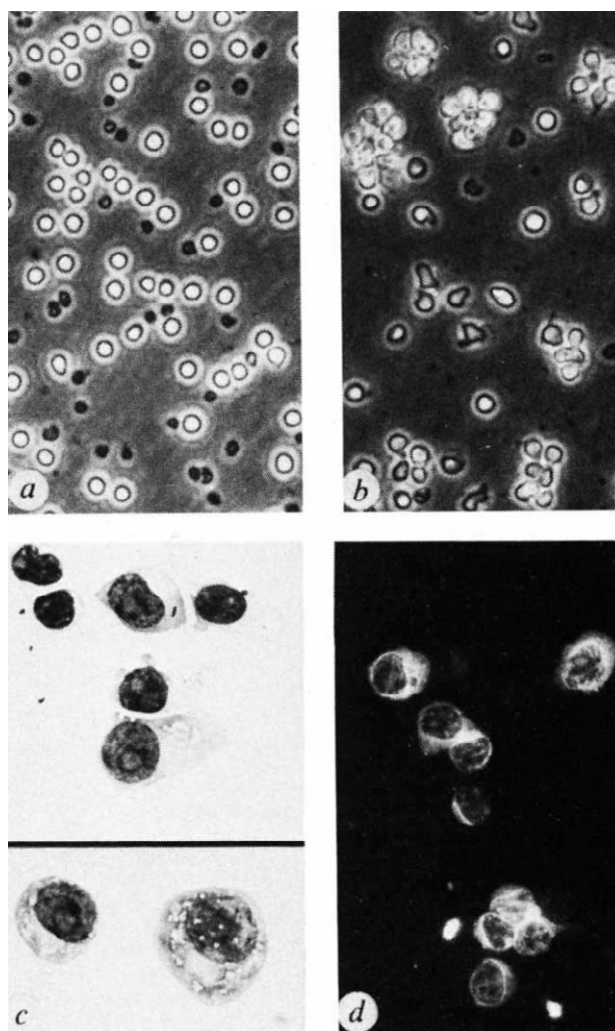


Fig. 3 Morphology of CLL cells (patient AK) cultured for three days in the presence of 1.6×10^{-7} M TPA. *a* and *b*, Cultures of living untreated and TPA-treated cells, respectively ($\times 220$). *c*, TPA-treated cells stained with MGG ($\times 660$). *d*, Immunofluorescence of TPA-treated cells stained for cytoplasmic μ chains ($\times 450$).

lymphoblastoid and plasmacytoid cells, but not the small fraction of small lymphocytes, stained strongly for C-Ig (Fig. 3d and Table 1). In control experiments, TPA-induced CLL cells (day 3-4) from the four patients were stained for C-Ig using mono-specific antisera against the immunoglobulin heavy chains μ , δ , γ , α and ϵ as well as κ and λ light chains. The C-Ig was always of the same isotype as the S-Ig detected on the corresponding freshly separated blood cells, whereas irrelevant

CLL cells, as reflected by heavy-chain (μ) variations. On average 5.5% of the fresh CLL cells contained cytophotometrically detectable amounts of C-Ig, and the proportion of such cells increased only slightly in the control cultures during 4 days. However, in cultures containing TPA there was a rapid increase in the percentage of C-Ig-positive lymphoid cells from (on average) 49% after 1 day to 92% after 3-4 days of culture. The relative amount of μ chains increased two- to ninefold in TPA-treated CLL cells as compared with untreated cells. Under the influence of TPA, the S-Ig of CLL cells behaved reciprocally, as the proportion of S- μ chain-positive cells decreased from (on average) 90 to 9% together with a decrease in fluorescence intensity. However, a slight to moderate reduction in the percentage of S- μ chain-positive cells was also seen in untreated control cultures (Table 1).

The poor *in vitro* proliferative response of CLL cells to various mitogens¹⁶⁻¹⁸ has previously led to the conclusion that CLL represents a clonal expansion of malignant B-lymphoid cells arrested at the stage of differentiation roughly corresponding to that of small resting lymphocytes. However, Fu and colleagues¹⁹ recently studied two CLL patients expressing monoclonal serum Ig components idiotypically identical with the S-Ig of the CLL cells. When assisted by allogeneic helper T cells or Epstein-Barr virus (EBV), a fraction of the CLL cells matured into monoclonal, Ig-secreting plasma cells. A variable *in vitro* differentiation can also be induced in some ordinary CLL tumour populations using polyclonal B/T lymphocyte activators or EBV²⁰.

The present study demonstrated that TPA, which has been previously found to be effective in inducing differentiation of mouse⁷ and human⁷⁻¹¹ myeloid and monocytic¹² malignant cells, also effects CLL cells. The sequence of changes in the immunoglobulin expression and the ultrastructure of TPA-treated CLL cells is similar to that observed during differentiation of normal B cells. It is, therefore, possible that TPA-induced differentiation of CLL cells may be a useful model for human B-cell differentiation *in vitro*. In contrast to the EBV- or mitogen-induced differentiation, TPA seems to affect the whole tumour cell population, as more than 90% of the CLL cells changed both morphologically and with respect to C- and S-Ig expression. A second difference between EBV/mitogen and TPA as differentiation inducers in CLL cells seems to be that differentiation with TPA is associated with concomitant inhibition of DNA synthesis, as suggested by preliminary thymidine incorporation studies. Biosynthetic studies with TPA-induced CLL cells using a plaque assay and immunoprecipitation²¹ indicate a cytoplasmic accumulation but only a minimal secretion of monoclonal immunoglobulin. Possibly the B cells do not reach the degree of maturation necessary for substantial immunoglobulin secretion. Alternatively, TPA may block secretion perhaps as a result of the inhibition of DNA synthesis.

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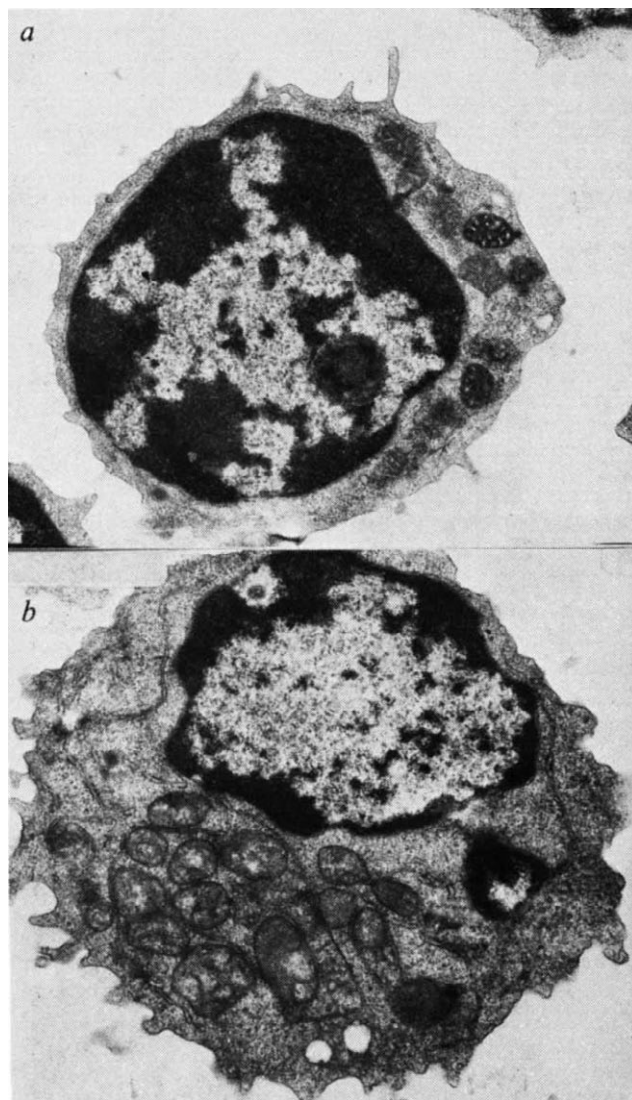


Fig. 4 TPA-induced ultrastructural maturation of CLL cells. Lymphocytes cultured for three days in the absence (a) and presence (b) of TPA ($\times 18,500$). Note the increase in the number of mitochondria, the amount of endoplasmic reticulum and the higher cytoplasmic:nuclear ratio in the TPA-treated cells.

antisera stained only 0-1% of the cells weakly (data not shown). Therefore, TPA clearly induced the tumour cell population and not merely contaminating normal cells in these experiments. The TPA-induced ultrastructural changes in CLL cells (Fig. 4) consisted of an increased cytoplasmic:nuclear ratio together with increased amounts of mitochondria, endoplasmic reticulum and Golgi structures.

Table 1 shows the time-dependent TPA-induced changes in the expression and amounts of monoclonal C-Ig and S-Ig of

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Differential expression of H-2 gene products in tumour cells is associated with their metastatogenic properties

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Many neoplasms seem to be heterogeneous in nature^{1,2}, producing metastases by pre-existing variant cells^{3,4} with inherent biochemical and biological properties. The survival and proliferation of metastatic cells depend on various biological properties, such as enzymes which degrade basement membranes⁵, resistance to various host defence systems^{6,7}, association with host cellular components⁸, adhesiveness⁹ and expression of certain membrane glycoproteins^{10,11}. Recent studies have indicated that metastatic cells may differ from the local tumour cells in the expression of immune recognizable membrane-associated antigens^{12,13}. Such antigenic differences may result from an immunoselection of cells with distinct antigenic properties due to a specific immune response evoked against the local tumour. In view of the role of the major histocompatibility complex (MHC) system in controlling and restricting the function of immune effector cells against modified self-components^{14,15}, one could assume that the modulation of the expression of MHC-encoded antigens on the membrane of tumour cells influenced the interclonal relationship within a local heterogeneous tumour cell population and the subsequent generation of metastasis. The modulation of the expression of H-2 antigens on several murine tumours is well documented^{16,17}; however, practically no attempts were made to relate H-2 modulation with invasiveness. We now describe principal differences in the expression of H-2 parental haplotypes between a local F₁ methylcholanthrene-induced tumour and its descendant pulmonary metastases. These results suggest that both the expression and the immunogenicity of MHC products strongly influence the immune relationship between the tumour and the host's immune system, thus determining the generation and dissemination of metastases.

Mouse (T10) methylcholanthrene-induced sarcoma cells were serially transferred in syngeneic (C3HeB×C57BL/6)F₁ mice (H-2^b×H-2^k). Animals carrying subcutaneous solid T10 tumours regularly show the appearance of metastatic nodules in the lungs¹⁸. Because we intended to probe quantitative and qualitative differences in the membrane expression of murine MHC-encoded antigens on the local tumour and its metastatic descendants, local T10 (L-T10) and metastatic T10 (M-T10) cells were analysed for the membrane expression of both parental H-2^b and H-2^k haplotypes. The membrane expression of H-2 antigens was analysed using fluorescence serology carried out with the fluorescence-activated cell sorter (FACS-II). The use of the FACS allowed us to screen only for cells expressing high density of the relevant H-2 antigen. Such cells will be referred to as brightly stained cells. Solid tumours originating from subcutaneous transplants of L-T10 or M-T10 cells in (C3HeB×C57BL/6)F₁ mice were trypsinized and single-cell suspensions analysed for the relative number of cells stained either with anti-H-2^k or with anti-H-2^b antisera. From the comparison of the relative number of tumour cells expressing H-2^b and H-2^k antigens (data outlined in Table 1) and of the fluorescence distribution of M-T10 and L-T10 cells stained with either anti-H-2^b or anti-H-2^k antisera (Fig. 1), a clearcut difference was observed in the membrane expression of the parental H-2 haplotype on L-T10 compared with M-T10 cells. The data suggest that L-T10 cells express mainly or exclusively the H-2^b parental haplotype whereas M-T10 cells express both the H-2^b haplotype (although at a lower level than L-T10 cells)

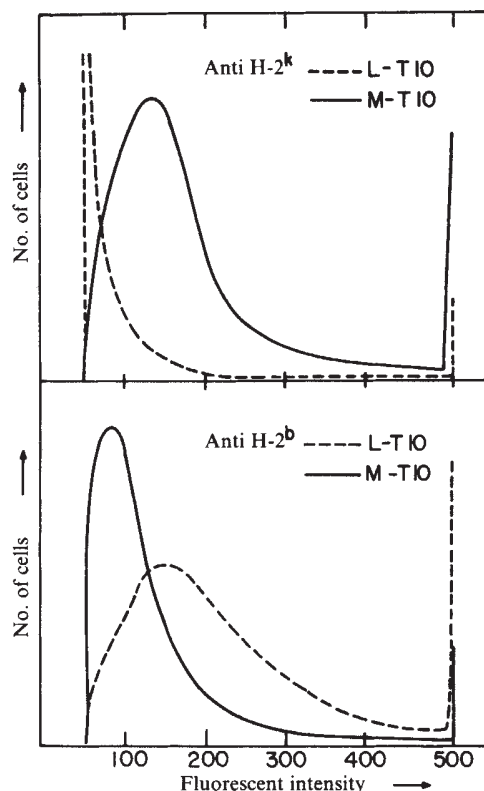


Fig. 1 Fluorescence distribution of L-T10 and M-T10 cells stained with anti-H-2^k or anti-H-2^b antisera. The same staining conditions were used as described in Table 1 legend.

and the H-2^k haplotype. These differential characteristics in H-2 expression were stable despite several serial transfers. In addition, different M-T10 clones (obtained from single metastatic nodules) showed identical staining patterns, implying the homogeneous nature of this variant. The H-2^k-encoded molecules expressed on the M-T10 cells consisted of the serological determinants encoded by the different subregions, as the M-T10 cells were stainable with anti-H-2^k antisera directed against both the H-2D and H-2K end products of the H-2^k haplotype (Table 1). Again, L-T10 tumour cells were found to be serologically negative when analysed with these specific antisera. To test whether the M-T10 cells were pre-existing variants in the local tumour population, the L-T10 tumour cells were cloned in semi-solid agar, and individual clones which were passaged in F₁ recipients were analysed for the presence of H-2^k or H-2^b

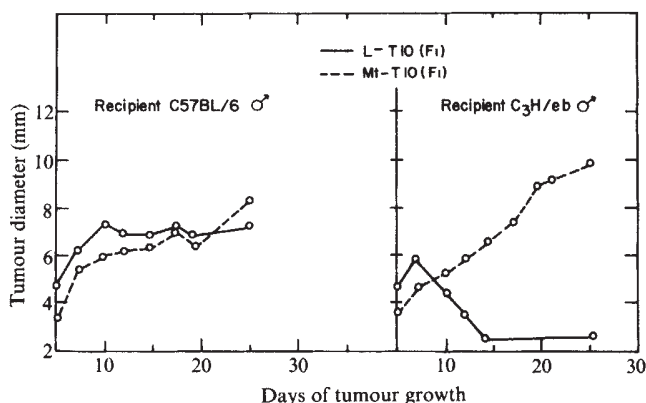


Fig. 2 The capacity of L-T10 and M-T10 tumour cells to grow in parental hosts. 10⁶ L-T10 or M-T10 cells were inoculated into the hind footpad of normal C57BL/6 or C3H/eb mice. At the indicated time intervals, the mean tumour diameter was recorded. Each group consisted of 10 mice.

membrane antigens. As shown in Table 2, the fluorescence analyses of 10 randomly chosen T10 clones revealed the presence of two clones (that is, IE7 and IB9) expressing both the H-2^k and the H-2^b parental haplotypes. The remaining clones were found to be H-2^k negative but H-2^b positive.

These last findings suggest that the original local T10 tumour consisted of a mixture of H-2^k-positive and H-2^k-negative cells, with a predominance of H-2^k-negative cells. To approach the question of whether the H-2^k-positive T10 tumour cells were identical in nature to the M-T10 cells, the capacity of the different L-T10 clones to produce lung metastases was tested by injecting 10⁶ viable tumour cells intravenously (i.v.) and 2 weeks later examining the organs for the appearance of tumour nodules. The data outlined in Table 2 clearly indicate that the H-2^k-positive clones are potentially able to survive in the peripheral blood stream and to develop subsequently in one organ, the lungs. No such features were observed with the H-2^k-negative clones. These two concomitant characteristics—membrane expression of the H-2^k parental haplotype and homing to the lung tissue—were stable, remaining unchanged even after 10 serial passages in syngeneic F₁ mice.

Additional evidence for the ability of H-2^k-positive T10 tumour cells to circumvent the host's resistance mechanisms was obtained by analysing the growth capacities of L-T10 and M-T10 cells in parental recipients. When 10⁶ viable L-T10 cells or M-T10 cells were grafted intra footpad to C57BL/6 (H-2^b) mice or C3H/eb (H-2^k) mice, differential growth patterns were observed, depending on the origin of the T10 tumour cells (Fig. 2). M-T10 cells were found to grow both in an H-2^b and in an H-2^k environment, whereas L-T10 cells grew only in H-2^b mice

Table 1 Parental H-2 haplotype expression on L-T10 and M-T10 tumour cells

Expt no.	Tumour cells (origin)	Relative no. of cells stained with:			
		αH-2 ^b	αH-2 ^k	αH-2D ^k	αH-2K ^k
1	L-T10	85	0	0	0
	M-T10 ₁	44	71	74	42
	M-T10 ₂	45	78	80	38
	M-T10 ₃	40	71	60	45
2	L-T10	93	24	8	7
	M-T10	55	85	86	50
	M-T10 ₁	61	86	87	42
	M-T10 ₃	40	82	73	53
3	L-T10	84	16	—	—
	M-T10 ₁	47	90	—	—
	M-T10 ₂	37	75	—	—

The T10 sarcoma tumours from the original local tumour (L-T10) or from different metastatic pulmonary nodules (M-T10_{1,2,3}) were maintained by subcutaneous transfers of 1 × 10⁶ viable cells in (C3Heb × C57BL/6)F₁ mice. Single cell suspensions from solid tumours were prepared by treatment of minced tumour tissue with a solution of 0.3% trypsin (hog pancreas; Nutritional Biochemicals). The fluorescence stainings of the L-T10 and M-T10 tumour cells were performed using the following experimental protocol: 0.1 ml cells (3 × 10⁷ cells ml⁻¹) in Dulbecco's balanced salt solution with 0.02% NaN₃ (DBSS) were incubated with 0.01 ml antiserum (optimal concentration) at 4 °C during 30 min followed by two washings with DBSS. After resuspension in 0.1 ml DBSS, 0.010 ml of an optimal dilution of fluorescein-labelled rabbit anti-mouse Ig (Nordic Immunological Laboratory) was added and the cells were incubated for 20 min at 4 °C. After two washings with DBSS, the cells were analysed on the FACS-II (Becton-Dickinson). A scatter window was set to eliminate dead cells and cell debris. The frequency and fluorescence profile of the stained cells were determined using a laser output of 400 mW, photomultiplier at 450 V and fluorescent gain 4. The anti-H-2 antisera were obtained using the following immunization protocol: C3H.SW (H-2^b) or C3H.DiSn (H-2^k) were immunized intraperitoneally with 10⁸ spleen cells of either C3H.DiSn or C3H.SW, respectively, weekly, for 4 weeks. Five days after the last injection the mice were bled and the obtained antisera checked for specificity using spleen cells and hydrocortisone-resistant thymocytes of different H-2^k or H-2^b congenic strains. The staining experiments outlined here were repeated on three successive serial transfers of L-T10 and M-T10 tumour cells (expts 1-3).

Table 2 Parental H-2 haplotype expression and metastatic potential of T10 clones

Expt no.	Tumour cells (origin)	Relative no. of cells stained with		Presence of nodules in lung	Experimental metastases after i.v. inoculation
		Anti-H-2 ^b	Anti-H-2 ^k		
1	L-T10	84	23	±	300
	T10 clone IC9	66	14	—	212
	IG2	67	16	—	214
	IIF3	73	8	—	241
	IG3	81	11	—	232
	IB9	93	65	+++	655
	IE7	97	73	+++	759
	IF7	94	17	ND	ND
	IID6	77	14	ND	ND
	IID9	82	15	ND	ND
2	L-T10	87	12	±	241
	T10 clone IE7	91	93	+++	765
	IID6	77	10	—	214
	IB9	73	74	+++	704
	IC9	71	7.8	—	205
	L-T10	82	13	±	256
3	T10 clone IE7	80	77.4	+++	960
	IB9	89	94	+++	803

T-10 cells were cloned in soft agar as previously described²⁰. Briefly, tumour cells were seeded in soft agar medium (0.3%) on a harder agar (0.5%) medium supplemented with 15% FCS. After 10-14 days of incubation colonies were picked, expanded in liquid medium and transplanted subcutaneously into syngeneic F₁ mice. Single cell suspensions obtained after trypsin treatment of the different T10 tumours were analysed for the expression of H-2 antigens and for the capacity to form experimental metastases. The fluorescent stainings with anti-H-2^b and anti-H-2^k antisera on L-T10 tumour cells and different T10 clones were performed as described in Table 1 legend. The metastatic potential of different T10 clones was assessed by injecting 10⁶ cells i.v. and 2-3 weeks later mice were killed and examined macroscopically for evidence of metastases to the lungs (—, no metastases; ±, <10 nodules, +, +, +, extensive involvement by tumour, numerous metastases). The level of infiltration was estimated by weighing the lungs (weight of normal lung = ±200 mg). The membrane expression of the parental H-2 haplotypes and the metastatic potential were repeated on some of the T10 clones obtained after different serial transfers (expt 1 = first transfer, expt 2 = 5th transfer; expt 3 = 10th transfer). ND, not done.

and were completely rejected in H-2^k mice. This result suggests that the presence of H-2^k antigens on the surface of T10 tumour cells somehow impairs semialloantigenic recognition.

Using a methylcholanthrene-induced tumour in (DBA/2 × A)F₁ mice (MDAY), Kerbel *et al.*¹⁹ recently reported that serial transfers of MDAY tumour cells through the parental DBA/2 mice led to the selection or formation of a putative H-2K^k loss variant, with highly metastatic characteristics. This finding and our present results may suggest that the modulation in the expression of MHC products generates in certain instances neoplastic cells with higher ability to resist immune destruction, resulting in increased survival in the peripheral blood, before seeding in distant anatomical locations.

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Quantification of noradrenaline iontophoresis

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Several problems are encountered when iontophoresis^{1,2} is used to study the effects of putative neurotransmitters. The most significant is that it is not usually practical to estimate the concentration of drug obtained at the tip of the microelectrode by a current of a given strength. The usual methods, albeit rarely used, include measurement of transport numbers^{3,4}, the use of ion-sensitive microelectrodes^{5,6} and quantitative fluorescent microscopy⁷. With the exception of the ion-sensitive microelectrodes developed for acetylcholine⁵, these techniques are elaborate and time consuming, and cannot be routinely applied to every electrode used. Furthermore, conventional multibarrel microelectrodes have high-impedance recording barrels and thus often display low signal-to-noise ratios when recording single-cell activity, the noise being increased during iontophoresis. We describe here a technique which largely overcomes the problem of low spike signal-to-noise ratio in conventional multibarrel electrodes, and which, unlike the latter, also allows precise determination of the concentration of noradrenaline in the environment of the cell, which affects its excitability. The recording and iontophoretic properties of these electrodes have been described previously⁸. The use of these electrodes to quantify precisely iontophoretically noradrenaline by adapting polarographic techniques is described.

The high-speed polarographic technique was developed from systems being used for evaluating catecholamine levels in the central nervous system^{9,10}. Our system differs principally in that it is some hundreds of times faster, is carried out at the single-cell, rather than macrocellular, level, and combines iontophoresis and single-cell recording. Full details of the methodology will be presented elsewhere; however, the principal features and a block diagram of the system (Fig. 1) are described here.

Three barrel microelectrodes were constructed in which one barrel contained a carbon fibre (outer diameter 7–8 µm), protruding 15 µm from the glass, and etched to a 1-µm point^{11,12}. The other two (fluid-filled) barrels had outside diameters of 2–3 µm. The carbon fibre electrode was connected via saline solution in the stem of the pipette to a current-to-voltage 'virtual-ground' field effect transistor (FET) operational amplifier. One other (reference) barrel was filled with 2 M NaCl, and connected to a voltage-follower FET. The third barrel was

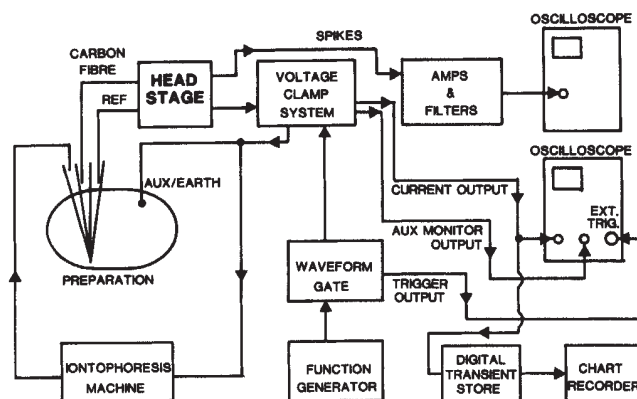
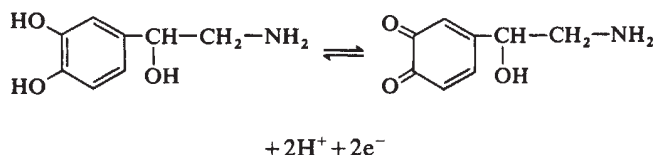


Fig. 1 A block diagram of the apparatus used for spike recording and high-speed polarography. Switches are incorporated in the headstage and voltage-clamp systems. For extracellular single-unit (spike) recording, the signal from the carbon fibre-containing microelectrode barrel (CARBON FIBRE) is switched into a low-noise voltage follower in the headstage and then amplified and filtered in a conventional way. The auxiliary electrode (AUX/EARTH) is switched to ground. For polarography, a triangular waveform (see Fig. 2a) is obtained from a function generator and a waveform gate. This is fed into the preparation using the auxiliary electrode. The voltage at the tip of the microelectrode is monitored by the reference barrel (REF) and via a voltage-clamp system makes the voltage at the tip exactly follow the applied waveform. The current flow to ground through the carbon fibre caused by this voltage pattern is displayed on an oscilloscope, and may be output to a chart recorder through a digital transient store. A second channel of the oscilloscope monitors the voltage appearing at the auxiliary electrode.

filled with 0.1 M noradrenaline (NA) in dilute HCl (pH ~ 2) for iontophoresis.

For *in vitro* calibration, the electrode tip was lowered into a beaker of 0.9% NaCl. Also dipping into the saline was a chlorided silver wire (the auxiliary electrode). The reference electrode was connected in a negative feedback loop with the auxiliary electrode, such that the auxiliary electrode would supply current to force the potential at the tip of the carbon microelectrode (monitored by the reference electrode) to follow any desired input voltage pattern. The current flow through the carbon fibre caused by these voltage changes was measured and displayed on an oscilloscope and printed out on a chart recorder via a digital transient store. A triphasic triangular waveform was used as the input voltage pattern. It was obtained by gating the output of a function generator. This waveform had a peak voltage of ±0.7 V, a duration of 15 ms and was repeated twice per second (Fig. 2a). The precise current flow in the carbon electrode caused by this voltage pattern was determined by the impedance of the electrode-saline system¹¹, and varied slightly from electrode to electrode, but was always similar to that shown in Fig. 2b.

When NA was added to the saline in the beaker, electro-oxidation of the amine occurred during the part of the cycle when the carbon fibre was anodic, according to the formula



It is generally accepted that the electrons liberated are taken into the working electrode (carbon fibre) and cause an increase in anodic current, and that subsequent reversal of potential causes a reduction of the quinone product back to NA, producing a corresponding increase in cathodic current⁹. Figure 2c

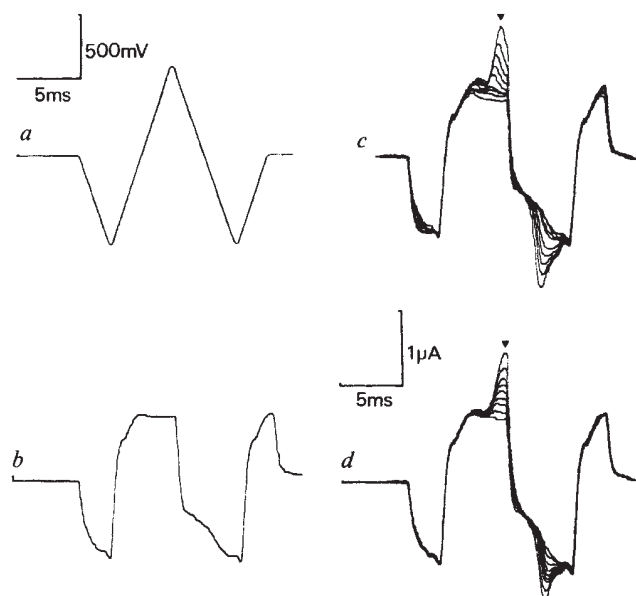


Fig. 2 Polarographic currents recorded with carbon fibre microelectrodes. *a*, The voltage pattern applied to the carbon fibre tip of the microelectrode; *b*, shows the resultant current flow through the carbon fibre produced by this voltage pattern, when the microelectrode tip was immersed in 0.9% saline in a beaker; *c* shows a set of nine such polarographic current sweeps from a microelectrode in saline and eight different concentrations of NA in saline. The concentrations were 2.8×10^{-4} M for the largest trace, and then 1.4×10^{-4} M, 7, 5, 3, 2 and 1×10^{-5} M, and finally 5×10^{-6} M, for the second-smallest trace. The 'peak incremental current' for each concentration was measured at the time marked by the filled triangle. This was the extra current over the saline current caused by the oxidation of NA. *d* shows a set of current sweeps obtained with the microelectrode in saline using nine different levels of iontophoretic eject (tip positive) current through the NA-containing barrel of the microelectrode. They were 0, 10, 20, 40, 60, 80 and 120 nA. Scale in *a* applies only to *a*; scale in *d* applies to *b*, *c* and *d*.

shows polarographic current sweeps in a series of different NA concentrations. Increasing concentrations of NA produced progressively increasing increments on the sweep at the peaks of oxidation and reduction. The reason for using a fast-gated triangular waveform was that this voltage pattern minimized build-up of reaction product and consequent poisoning of the electrode.

These experiments were done with a 'retain' (tip negative) iontophoretic current on the NA barrel. By reducing the retain current to zero, it was found that most of the NA-containing iontophoresis barrels showed appreciable 'leak' or passive diffusion of NA. By applying polarographic current sweeps at 2–10 Hz during iontophoretic eject currents of various levels, one could measure the concentrations of NA at the electrode tip. To establish adequate retain current, retain current was increased during polarographic pulses at 2 Hz. When the catecholamine signal disappeared, and further increase in retain current gave no change in signal, the retain current was considered adequate. For establishing steady levels of iontophoretically ejected catecholamine, we waited until the level of catecholamine signal maximally stabilized at a constant iontophoretic current. This 'lag time' varied in different electrodes and will be reported elsewhere. Signals from successive sweeps in these conditions invariably showed no detectable variations in amplitude, even when iontophoresis was continued for up to 30 min. Longer periods were not investigated.

Figure 2*d* shows a set of current sweeps made during NA iontophoresis using several different levels of ejection current.

The similarity between Fig. 2*c* and *d* is apparent. From the data in Fig. 2*c*, a calibration curve was constructed relating peak incremental current (measured at the points indicated by filled triangles) to the molar concentration of NA. From this curve and the data in Fig. 2*d*, a second curve relating iontophoretic eject current to tip concentration of NA in steady-state conditions could be produced. This curve is shown in Fig. 3.

For *in vivo* studies, the electrode was inserted into the primary somato-sensory cortex of urethane-anaesthetized rats. The auxiliary electrode was pushed into the retracted neck muscle. Figure 4 illustrates *in vitro* and *in vivo* data taken from another microelectrode. Figure 4*a* shows a set of current sweeps taken in different concentrations of NA in saline, ranging from 10^{-5} to 2×10^{-3} M NA. Figure 4*b* shows current sweeps during iontophoresis in saline at different currents ranging from 20-nA retain to 100-nA eject. Figure 4*c* shows two sweeps with 20-nA retain, one in saline and the other *in vivo* (1,000-μm down in the rat cortex). Apart from a slight increase in cathodic current *in vivo*, the sweeps are identical. Finally, Fig. 4*d* shows a set of current sweeps *in vivo* using the same iontophoretic eject currents as in Fig. 4*b*, taken after 60 s of iontophoretic eject current for each sweep. The catecholamine signals can be seen to be bigger *in vivo* than *in vitro*, presumably due to restricted diffusion of NA *in vivo*. In other cases, *in vivo* NA levels were less than *in vitro* ones, or fluctuated from second to second despite steady iontophoretic ejection, possibly reflecting variations in active noradrenergic uptake. Anyway, the signal shows the moment-to-moment variation in catecholamine levels at the investigated cell.

By switching the auxiliary electrode connection to ground and the carbon fibre electrodes into a low-noise voltage follower, extracellular voltage spikes were recorded from the investigated cortical neurone. The response of such neurones to iontophoretic NA was complex and will be reported elsewhere. Used as recording microelectrodes, carbon fibres had significantly lower noise than micropipettes and could distinguish axon and soma-dendrite spikes by waveshape^{8,13,14}.

The technique of high-speed polarography using carbon fibre microelectrodes should have wide application in the study of the neurobiology of electroactive neurotransmitters. The method combines three techniques at the single-cell level—iontophoresis, recording of unit activity changes elicited by the

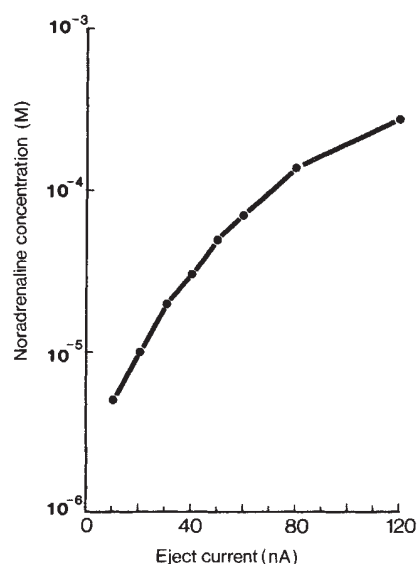


Fig. 3 Calibration curve relating the concentration of NA present at the tip of a multibarrel microelectrode immersed in saline to the level of steady iontophoretic ejection current through a NA-containing barrel. (The curve is constructed from data shown in Fig. 2*c*, *d*).

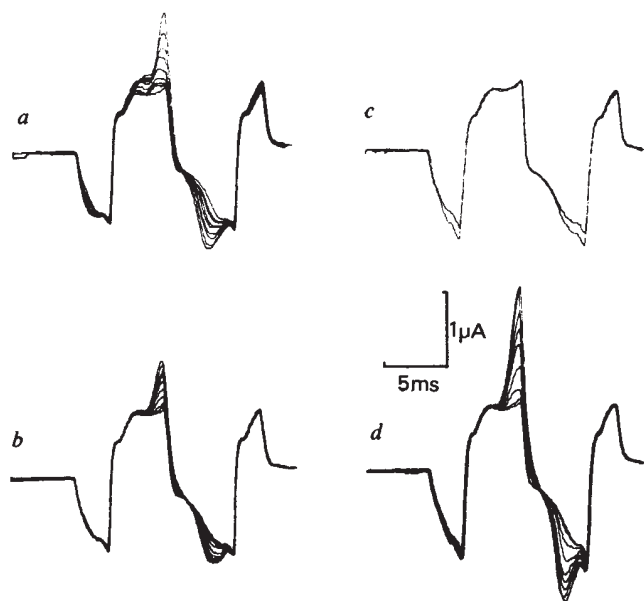


Fig. 4 Polarographic current sweeps *in vitro* and *in vivo*. *a*, A series of current sweeps for microelectrode in different concentrations of NA in saline, from 10^{-5} to 2×10^{-3} M. *b*, The same microelectrode in saline using a series of different NA iontophoretic ejection currents. Current levels were, from smallest to largest, 20 nA tip negative, 0 mA (passive leak), 20, 40, 60, 80, 100, 120, 160 nA tip positive. *c*, Control current sweeps (20-nA retain) in saline and in the cerebral cortex of an anaesthetized rat. The current *in vivo* is slightly larger on the cathodal (reducing) part of the cycle. *d*, A set of sweeps in the cerebral cortex using the same series of steady iontophoretic NA ejection currents as in *b*. In *b* and *d* the sweeps were made 1 min after the start of iontophoresis.

iontophorese substance and accurate measurement of the concentration of the iontophorese substance. The sensitivity of the technique allows concentrations of 5×10^{-8} M NA and dopamine to be distinguished from background signal¹⁵. In comparison, other polarographic techniques^{10,16,17} do not have the facility for iontophoresis, unit activity recording or electrode calibration *in vitro* before use *in vivo*. Finally, the time course of this polarographic recording technique (15 ms) approaches the time courses encountered during changes in neuronal activity in the nervous system. By using suitable high-speed switching between recordings of unit spike activity and polarographic measurement of catecholamine concentration, studies of the time course of responses in relation to known concentrations of drug in the central nervous system, which has previously been impossible, should now become feasible.

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Vitamin E modulates the lipoxygenation of arachidonic acid in leukocytes

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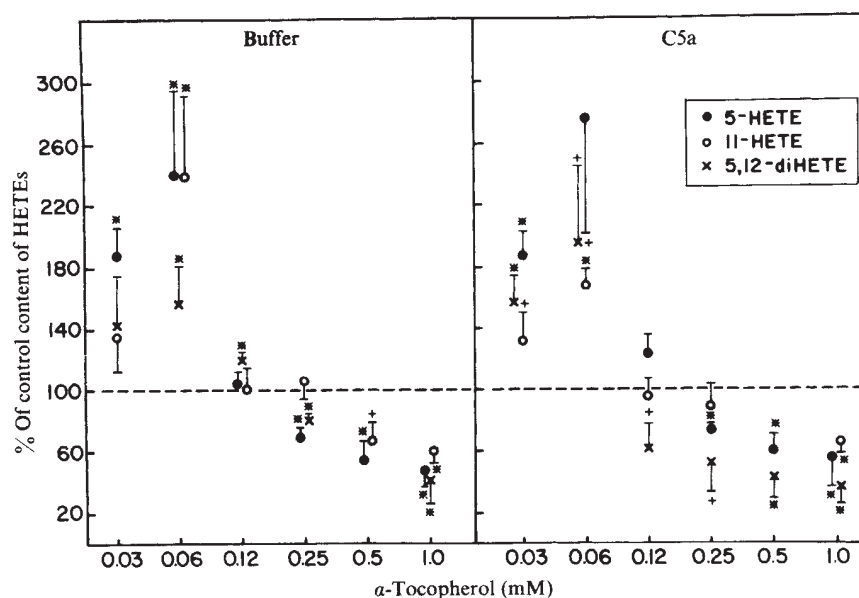
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The arachidonic acid released from cellular phospholipids of specifically stimulated platelets and leukocytes is oxygenated enzymatically by two major pathways^{1–5}. A complex cyclo-oxygenase converts some of the free arachidonic acid to labile endoperoxides that are transformed to prostaglandins, thromboxanes and prostacyclin (PGI₂). Lipoxygenases convert part of the arachidonic acid to unstable hydroperoxy-eicosatetraenoic acids (OOHETEs) that are transformed to monohydroxy-eicosatetraenoic acids (HETEs), oligohydroxy-eicosatetraenoic or -eicosatrienoic acids such as di-HETEs and tri-HETEs, and, in some instances, more complex humoral mediators, including slow-reacting substances^{5–9}. Both the nature of the HETEs and the ratio of the HETEs to the cyclo-oxygenase products are specific characteristics of each type of cell^{2,5}. In human neutrophils, the sum of the lipoxygenase products 5-HETE, 11-HETE and 5,12-di-HETE substantially exceeds the total amount of PGE₂ and other cyclo-oxygenase metabolites that are generated concurrently^{3,4}, and the endogenous lipoxygenase products regulate neutrophil function. The present data indicate that vitamin E (α -tocopherol) bidirectionally modulates the activity of the lipoxygenase pathway of human neutrophils *in vitro*. Normal plasma concentrations of α -tocopherol enhance the lipoxygenation of arachidonic acid, whereas higher concentrations of α -tocopherol exert a suppressive effect that is consistent with its role as a hydroperoxide scavenger.

As extracellular mediators, the HETEs stimulate polymorphonuclear leukocyte random and chemotactic migration^{10–12}, enhance the expression of C3b receptors¹² and increase the intra-leukocyte concentration of cyclic GMP (ref. 13). As the bulk of HETEs generated by human neutrophils and eosinophils is retained in the cellular membranes, whereas the PGE₂ produced is secreted, it has been suggested that the HETEs are functionally critical intracellular constituents^{14,15}. In support of this, preincubation of neutrophils or eosinophils for 30–60 min with specific lipoxygenase inhibitors (for example, nordihydroguaiaric acid (5 μ M)) was observed to result in both significant depletion of cellular HETEs and suppression of random and chemotactic migration. The suppression of migration was reversed by the addition of purified exogenous HETEs to neutrophils and eosinophils deficient in endogenous HETEs as a result of pretreatment with lipoxygenase inhibitors^{14,15}.

The levels of endogenous HETEs in neutrophils not stimulated by a chemotactic factor were enhanced a mean of two- to threefold by preincubation for 20 min at 37 °C with 0.03–0.12 mM α -tocopherol and were suppressed substantially by 0.5–1 mM α -tocopherol ($n = 3$). Neutrophils incubated with a chemotactic concentration of C5a exhibited mean increases of 2-, 1.5- and 2.5-fold, respectively, in the intracellular content of 5-HETE, 11-HETE and 5,12-di-HETE ($n = 4$), and α -tocopherol similarly modulated the enhanced level of HETEs in a concentration-dependent manner. In each instance, the increases and decreases were greatest for 5-HETE, whereas those of 5,12-di-HETE proved to be most resistant to suppression by 0.5–1 mM α -tocopherol. The quantities of HETEs in the extracellular fluid were consistently less than 20% of those in the neutrophils, but the alterations elicited by α -tocopherol paralleled those in the corresponding neutrophil pellets. To establish that the mechanism of action of α -tocopherol was not a function of changes in the rate of mobilization of arachidonic acid from

Fig. 1 Effects of α -tocopherol on the lipoxygenation of exogenous arachidonic acid by human neutrophils. Portions of 1×10^8 purified neutrophils were preincubated in 6 ml of Hanks' solution/0.1% ovalbumin (w/v), without or with various concentrations of highly purified α -tocopherol (Supelco) for 20 min at 37 °C. Neutrophils were then washed twice, resuspended in 6 ml of the corresponding buffer without or with the same concentration of α -tocopherol and incubated for 15 min at 37 °C with 160 μ g of arachidonic acid and 100 μ l of buffer (left-hand frame) or 100 μ l of the purified 15,000-molecular weight chemotactic fragment of the fifth component of complement, designated C5a (right-hand frame)¹²⁻¹⁵. The neutrophils were sedimented at 100g and the supernatant and neutrophil pellet, which had been resuspended in 6 ml of Hanks' solution and sonicated, were acidified to pH 4 with 2 M citric acid. After the addition of 40,000 c.p.m. of 3 H-12-L-HETE^{14,15} to each sample, the HETEs were extracted from each supernatant and sonicated with two 6-ml portions of chloroform/methanol (2:1, v:v) and two 3-ml portions of ether. The HETEs in the pooled extracts were separated from α -tocopherol, resolved and purified by sequential silicic acid chromatography and reversed-phase HPLC^{14,15} and quantified by optical density at 235 nm (mono-HETEs) or 280 nm (5,12-di-HETE) with a correction for recovery according to the yield of 3 H-12-L-HETE. The range of intracellular content of 5-HETE, 11-HETE and 5,12-di-HETE in control neutrophils (100%) was 3,711–5,841, 1,833–3,271 and 594–1,071 ng per 10^8 neutrophils, respectively, in buffer alone and 6,914–11,046, 3,774–5,650 and 1,468–2,348 ng per 10^8 neutrophils with C5a. Each data point and bar are the mean $\pm$ s.d. for four experiments. The level of statistical significance of the difference between each value and the corresponding control value were determined by a standard two-sample *t*-test and are depicted by * $P < 0.01$ and † $P < 0.05$.



phospholipids, the studies were repeated using 160 μ g of exogenous arachidonic acid per 10^8 neutrophils, of which over 90% was taken up by the neutrophils to provide a quantity that exceeded by approximately twofold the total amount in the native cellular pool^{14,15}. Although the introduction of exogenous arachidonic acid augmented the content of intracellular HETEs by nearly 10-fold, the bidirectional effects of α -tocopherol (Fig. 1) were similar in magnitude to those elicited in the absence of exogenous arachidonic acid. The optimal increase in the content of each of the HETEs was evoked by 0.06 mM α -tocopherol and maximal suppression was achieved by 1 mM α -tocopherol.

As α -tocopherol reacts specifically with OOH-groups, it was also anticipated that the generation by neutrophils of 5-OOHETE and other OOHETEs would be influenced by alterations in the cellular level of α -tocopherol (Table 1). The ratio of the quantities of 5-OOHETE to 5-HETE was increased more than twofold by 0.03 mM α -tocopherol and was suppressed to ~30% of control values by 0.5 mM α -tocopherol in the absence of a specific agonist. Despite the 5.1-fold higher mean levels of 5-HETE in neutrophils exposed to the calcium ionophore A23187, the same concentrations of α -tocopherol exerted effects on the ratio of 5-OOHETE to 5-HETE that were of similar magnitude to those observed for neutrophils in buffer (Table 1). Alterations by α -tocopherol in the ratio of OOHETE to HETE secreted by neutrophils would influence the net

activity of any concentration of the lipid factors as extracellular mediators. 12-OOHETE and 5-OOHETE elicit maximal neutrophil chemotactic responses at 4 μ g ml⁻¹ and 0.4 μ g ml⁻¹, respectively, that exceed by over 50% the maximal chemotactic responses to 10–20 μ g ml⁻¹ of 12-HETE and 1–2 μ g ml⁻¹ of 5-HETE¹⁰⁻¹³. Similarly, both OOHETEs are more active than the respective HETEs in evoking an optimal neutrophil chemokinetic response. Not only are the OOHETEs 10-fold more potent than the HETEs in augmenting the intra-neutrophil concentration of cyclic GMP, but also the elevation achieved lasts for 30 min or longer, compared with between 10 and 20 min for that achieved by the HETEs.

α -tocopherol modulates human neutrophil migration *in vitro* at concentrations that affect the lipoxygenase pathway. The preincubation of neutrophils with 0.06 mM α -tocopherol for 20 min enhanced neutrophil random migration by 18–54% and chemotaxis to C5a by 29–106% ($n = 3$), whereas 1 mM α -tocopherol inhibited neutrophil random migration by 53–70% and chemotaxis by 32–56%, as assessed by a modified Boyden micropore filter assay¹²⁻¹⁵. The addition of nanogramme amounts of exogenous 5-HETE to 2×10^6 neutrophils depleted of endogenous 5-HETE and 11-HETE by prior treatment with lipoxygenase inhibitors, such as nordihydroguaiaretic acid, completely reverses the defect in neutrophil migration that is characteristic of the HETE-depleted neutrophils^{14,15}. Thus, it would be expected that the addition of 5-HETE to neutrophils preincubated in 1 mM α -tocopherol would reverse the inhibition of migration, if the sole abnormality were a depletion of endogenous HETEs. Preincubation of neutrophils with 1 mM α -tocopherol depleted the intracellular content of 5-HETE by a mean of 71% ($n = 3$), suppressed the ratio of 5-OOHETE to 5-HETE from a mean of 0.18 to 0.10 and concurrently inhibited random migration by 64% and chemotaxis to C5a by 52%. In contrast to the results with lipoxygenase inhibitor-treated neutrophils, however, the addition of 0.2–2.5 ng 5-HETE without or with 10–200 ng 11-HETE to 2×10^6 α -tocopherol-treated neutrophils failed to reverse the migration defects. The effects of α -tocopherol on human neutrophil function thus are not solely attributable to alterations in the cellular content of endogenous HETEs, and may also reflect changes in the concentrations of OOHETEs or non-lipoxygenase-related actions of α -tocopherol.

α -tocopherol serves an important role as 'biological antioxidant' by preventing the peroxidation of polyunsaturated fatty acid constituents of cellular membranes, including arachidonic

Table 1 Modification of the ratio of 5-OOHETE to 5-HETE in human neutrophils by α -tocopherol

Concentration of α -tocopherol (mM)	Buffer	A23187
0	0.16 $\pm$ 0.04*	0.17 $\pm$ 0.07
0.03	0.36 $\pm$ 0.12	0.32 $\pm$ 0.15
0.12	0.10 $\pm$ 0.06	0.12 $\pm$ 0.09
0.50	0.05 $\pm$ 0.01	0.08 $\pm$ 0.02

Portions of 3×10^8 purified neutrophils were preincubated in buffer without or with α -tocopherol, washed and resuspended in buffer of the same composition as described in Fig. 1 legend, with 160 μ g arachidonic acid per 10^8 neutrophils, and calcium ionophore A23187 at a final concentration of 5 μ M. After 10 min at 37 °C, the neutrophils were sedimented and the 5-OOHETE and 5-HETE extracted and purified as described in Fig. 1 legend. The ratio of 5-OOHETE to 5-HETE was determined by assessing the concentration of OOH-groups in the mixtures of the two compounds based on the conversion of triphenylphosphine to triphenylphosphine oxide and measurement of the quantity of triphenylphosphine oxide by gas-liquid chromatography²⁷.

* Each value is the mean $\pm$ s.d. of the results from three separate experiments.

acid¹⁶⁻¹⁹. A deficiency of α -tocopherol in rat or human neutrophils results in greater than normal release of hydrogen peroxide, augmented peroxidation of membrane lipids and impairment of chemotaxis and phagocytosis that are corrected rapidly by either the addition of α -tocopherol *in vitro* or the prior administration of several doses of α -tocopherol to the neutrophil donors²⁰. Although the optimal cyclooxygenation of arachidonic acid to prostaglandins in rabbit tissues requires physiological concentrations of α -tocopherol^{21,22}, the mechanism of this apparent facilitation of oxidation by α -tocopherol is unknown. The present data document for the first time the bidirectional modulation by α -tocopherol of the lipoxygenation of arachidonic acid to products which serve as both specific extracellular mediators and functionally critical intracellular constituents. The generation of endogenous neutrophil 5-HETE, 11-HETE and 5,12-di-HETE was significantly enhanced *in vitro* by 0.03 mM and 0.06 mM α -tocopherol, which approximate the normal human serum concentration of 0.01–0.05 mM (refs 23, 24). The neutrophil lipoxygenase pathway was inhibited significantly by levels of 0.25–1 mM α -tocopherol, which increase the content of α -tocopherol in platelets and leukocytes by up to 5–17-fold within 5 min of incubation *in vitro* and are obtainable *in vivo* by the ingestion of pharmacological doses of α -tocopherol^{23,24}. The mechanism of the enhancement of lipoxygenation of arachidonic acid in neutrophils by physiological levels of α -tocopherol is not related simply to alterations either in the formation of lipid hydroperoxides or in the liberation of endogenous arachidonic acid from phospholipids. The demonstration that the function of some acetylcholine receptors in phospholipid liposomes is

optimal only when α -tocopherol represents a substantial fraction of the total lipid content²⁵ and that α -tocopherol is required for the incorporation of excitability-inducing material into black lipid membranes²⁶, suggests that a normal content of α -tocopherol in neutrophil membranes also may be required for maximal expression of lipoxygenase activity.

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Differential repair of O^6 -methylguanine in DNA of rat hepatocytes and nonparenchymal cells

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Chronic administration of several chemical carcinogens to laboratory animals induces a variety of tumours which arise from specific cell populations within the liver. In the rat, diethylnitrosamine induces hepatocellular carcinomas¹, dimethylnitrosamine induces both angiosarcomas² and hepatocellular carcinomas³, vinyl chloride primarily induces angiosarcomas⁴, and 1,2-dimethylhydrazine induces malignant haemangioendotheliomas^{5,6}. One of the principal mechanisms thought to be involved in initiating carcinogenesis is the alkylation of specific sites on DNA, such as the O^6 position of guanine⁷. Previous investigations of alkylation and repair have, however, analysed DNA prepared from whole liver. This approach does not localize alkylation or repair capacity in the different cell types which give rise to neoplasia. Furthermore, although hepatocytes account for more than 90% of the liver's mass, they only comprise 60–70% of its cells⁸. The nonparenchymal cell (NPC) population, which consists almost entirely of endothelial and Kupffer cells, accounts for the remaining 30–40% and contains 10–20% of the DNA. Therefore, we decided to investigate the alkylation and repair of O^6 - and 7-methylguanine in the target and non-target cells following oral administration of 1,2-dimethylhydrazine. We report here that although initial alkylation was slightly less in NPCs, removal of O^6 -methylguanine was significantly slower. This led to a preferential accumulation of O^6 -methylguanine in NPC 24 h after administering a second daily dose. In contrast, 7-

methylguanine decreased at similar rates, resulting in a 28-fold greater O^6 -methylguanine/7-methylguanine ratio in the target cell population.

Administration of 1,2-dimethylhydrazine (DMH) at a dose of 3 mg per kg per day in the drinking water induces a high incidence of tumours arising from the vascular lining cells of the liver in BDIX rats. Investigations of DMH-induced alkylation and repair in whole liver have demonstrated that maximal DNA alkylation occurs within 12 h for doses ranging from 4 to 20 mg per kg (refs 9, 10). O^6 -Methylguanine is rapidly removed from DNA by an enzymatic process, whereas 7-methylguanine is lost at a slower rate by chemical depurination, leading to a decreasing O^6 /7-methylguanine ratio¹¹. This pattern is similar to that observed after the administration of dimethyl- or diethylnitrosamine¹². Nothing is known of the localization of methylated purines within the two major liver cell populations or the capacities of the different cells to repair O^6 -methylguanine.

Previous attempts to separate NPCs from hepatocytes have used density gradient centrifugation¹³ or the selective destruction of hepatocytes by pronase digestion¹⁴. Both these methods have serious limitations for use in a comparative study of alkylation and repair. When density gradient centrifugation is used, yields are inversely proportional to purity and many of the media used are hyperosmotic or mildly toxic. With pronase digestion, high yields of relatively pure NPCs can be obtained; however, destruction of the hepatocytes negates the possibility of studying two populations of cells from the same liver. In addition, the massive destruction of hepatocytes in the presence of the NPCs results in phagocytosis of hepatocellular debris by Kupffer cells⁸, possibly contaminating them with hepatocellular DNA. Elutriation centrifugation¹⁵, which separates mainly on the basis of cell volume, exploits the enormous difference between the volume of the hepatocyte and NPC⁸. This technique can be used to separate a mixed liver cell suspension derived from collagenase perfusion¹⁶ into populations of NPCs and hepatocytes with a purity of 95% or greater. The NPC population consists almost entirely of Kupffer and endothelial cells¹⁷. Bile duct cells remain in the filter leavings during the preparation of the mixed liver cell suspension. Purity of the populations can easily be monitored by light microscopy due to

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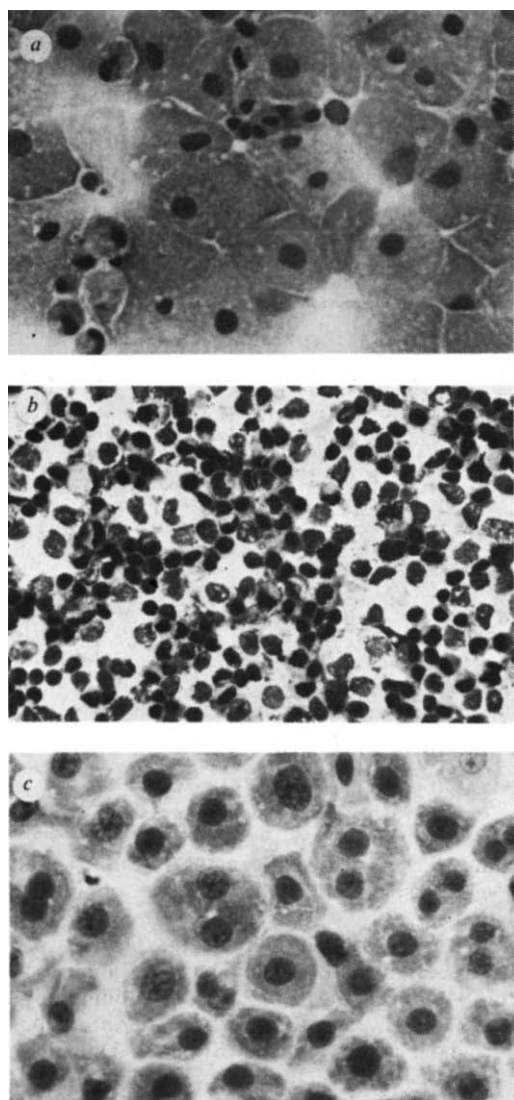


Fig. 1 Separation of a mixed liver cell suspension (MLCS) (a) into hepatocytes (b) and NPCs (c) by elutriation centrifugation (haematoxylin and eosin stain for hepatocytes, Wrights stain for NPCs $\times 400$). A MLCS was prepared as previously described¹⁶ by *in situ* perfusion of the liver with collagenase through the portal vein. The resultant MLCS was washed three times in HBSS without Ca^{2+} and Mg^{2+} at 4°C . After washing, the cells were resuspended in 20 ml of HBSS without Ca^{2+} and Mg^{2+} and slowly injected into the large mixing chamber (70 ml) of the Beckman JE-6 elutriation rotor containing a Sanderson separation chamber in a Beckman J2-21 refrigerated centrifuge equipped with a fine speed control. The rotor speed was held constant at 1,500 r.p.m., and the media flow rate varied. The MLCS was washed into the rotor at a flow rate of 18 ml min^{-1} until the separation chamber became approximately 50% full of retained hepatocytes. The flow of media was then diverted around the mixing chamber directly through the separation chamber and the retained hepatocytes washed until 200 ml of media containing the NPCs had been collected. The hepatocytes were then flushed from the rotor at a flow rate of 100 ml min^{-1} . After the separation chamber was emptied the flow rate was returned to 18 ml min^{-1} and the flow again routed through the mixing chamber washing in a second portion of the MLCS. This procedure was repeated four or five times until all the cells that could be washed from the mixing chamber had been separated. Yields varied from $2\text{--}5 \times 10^8$ NPCs and $6\text{--}11 \times 10^8$ hepatocytes per liver and were $\geq 95\%$ pure as determined by counting on a haemocytometer. The absence of bile duct epithelium in the NPC and hepatocyte fractions was confirmed using γ -glutamyl transpeptidase enzyme histochemistry.

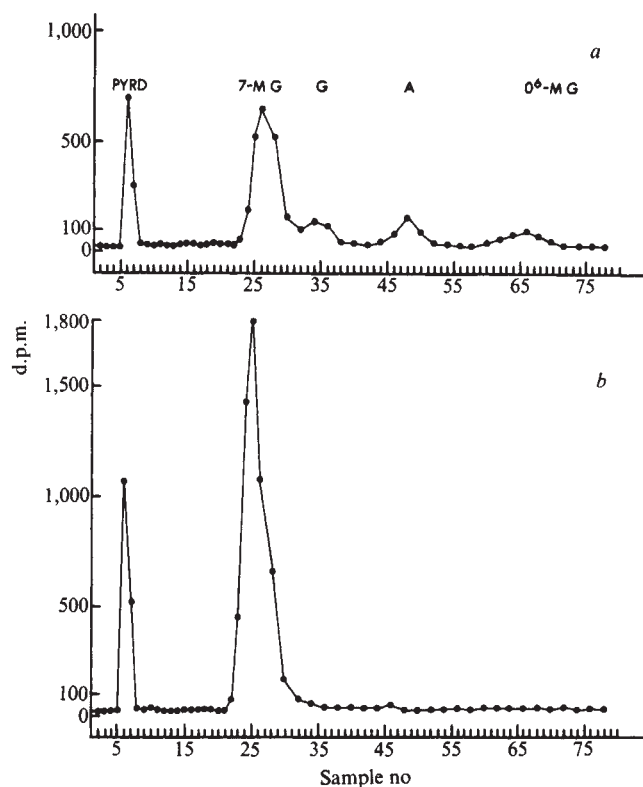


Fig. 2 Representative radiochromatograms of hydrolysed DNA from NPCs (a) and hepatocytes (b) of a rat killed 24 h after two daily doses of ^{14}C -DMH. DNA was extracted from the cell pellets by hydroxyapatite chromatography as previously described¹⁸ and the purines liberated by mild acid hydrolysis (0.1 M HCl at 80°C for 30 min). After hydrolysis the solution was made 0.01 M NaPO_4 , the pH adjusted with 1 M NaOH to 5.5, filtered and applied to a $45 \times 0.9\text{-cm}$ glass column containing 20 g (dry weight) of ultra fine Sephasorb HP (Pharmacia). The column was eluted with 0.01 M NaPO_4 buffer pH 5.5 at a flow rate of 1 ml min^{-1} (100 p.s.i.) using a Laboratory Data control HPLC pump. The concentrations of guanine and adenine were determined by integrating the area under the curve for their absorbance at 254 nm using a Shimadzu C-EIA integrator and a Uvicord S monitor. Samples of 3 ml were collected until the beginning of the guanine peak, after which 6-ml samples were collected. Two volumes of ACS scintillation fluid were added to each sample and they were counted for 10 min at a counting efficiency of 79% in a Searle mark III scintillation counter using channel 3. The concentrations of 7- and O^6 -methylguanine were expressed as a fraction of guanine and were calculated assuming that the specific activity of the alkylation products was one-half that of the carcinogen because only one labelled methyl group is available for alkylation¹⁹.

the large difference in size and morphology (Fig. 1). An additional advantage of elutriation centrifugation is that it can be performed at 4°C .

To quantify differences in alkylation and repair and to test for possible accumulation of alkylation products, nine male BDIX rats weighing 150–200 g were given an oral dose of ^{14}C -DMH (3 mg per kg, specific activity $7.5\text{--}8.0\text{ }\mu\text{Ci mmol}^{-1}$). Three animals were killed at 2 and 24 h, their livers perfused and the cells separated and frozen. The remaining three animals received a second dose of ^{14}C -DMH 24 h after the first dose, and were killed and their liver cells separated 24 h later. DNA from the different cell populations was purified by hydroxyapatite chromatography¹⁸, hydrolysed in 0.1 M HCl at 80°C for 30 min and the purines separated by gel filtration chromatography (Fig. 2).

The extent of the initial alkylation was slightly less in NPCs than in hepatocytes 2 h after carcinogen administration (Table 1). In contrast, after 24 h only 10% ($8.9 \pm 4.4\text{ pmol}$ of O^6 -methylguanine per μmol of guanine) of the initial amount of O^6 -methylguanine persisted in the hepatocytes, whereas 53%

Table 1 Alkylation of hepatocyte and nonparenchymal cell DNA following oral exposure of rats to ^{14}C -1,2-dimethylhydrazine

Cell type	No. of doses	Survival time (h)	Alkylation of DNA per 10^6 guanine			Metabolic incorporation of ^{14}C	
			$O^6\text{MG}$	7-MG	$O^6/7\text{-MG}$	Guanine (d.p.m. per μM)	Adenine (d.p.m. per μM)
Hepatocyte	1	2	$89.0 \pm 7.5^*$	$1,032 \pm 70$	0.086	ND†	ND
NPC	1	2	65.4 ± 8.4	783 ± 78	0.083	ND	ND
Hepatocyte	1	24	8.9 ± 2.5	753 ± 80	0.011	7.6 ± 7.7	18.3 ± 10.4
NPC	1	24	34.6 ± 0.7	525 ± 60	0.067	79.4 ± 46.8	78.7 ± 15.3
Hepatocyte	2‡	48	4.6 ± 3.6	$1,362 \pm 54$	0.003	8.1 ± 8.1	21.5 ± 13.6
NPC	2	48	56.3 ± 4.3	689 ± 90	0.085	375.6 ± 35.9	369.3 ± 53.8

* Mean $\pm$ s.e. of three rats.

† Not detectable.

‡ Second dose administered 24 h after the first dose.

(34.6 ± 1.1 pmol of O^6 -methylguanine per μmol of guanine) remained in the NPCs ($P < 0.01$, 1-tailed t -test). 7-Methylguanine decreased at a similar rate in the NPC (27%) and hepatocyte (30%) populations during the 24 h following oral administration of ^{14}C -DMH.

With 53% of the initial O^6 -methylguanine remaining in the NPCs, and assuming that previous and newly formed O^6 -methylguanine are repaired at the same rate, the amount of O^6 -methylguanine accumulating in the NPCs 24 h after a second dose of ^{14}C -DMH was estimated to be 53 pmol of O^6 -methylguanine per μmol of guanine. The observed value was 56.3 ± 4.3 pmol O^6 -methylguanine per μmol guanine. No accumulation of O^6 -methylguanine was detected in the corresponding hepatocytes whereas, in contrast, 7-methylguanine accumulated in both populations (Table 1). An additional indication of the marked differences in the two cell populations' capacity for repair of O^6 -methylguanine is demonstrated by comparing the $O^6/7$ -methylguanine ratios. These ratios were similar at 2 h, 6 times higher in the NPCs at 24 h and 28 times higher in the NPCs 24 h after administration of the second dose of ^{14}C -DMH (Table 1). As the 7-methylguanine was removed at a similar rate for both cell populations, the change in ratio clearly demonstrates that the hepatocytes were more efficient than the NPCs at removing O^6 -methylguanine from their DNA.

An additional difference observed between the two cell populations was the incorporation of ^{14}C from ^{14}C -DMH into normal purine bases via the C-1 pool. Incorporation of ^{14}C was much higher in the NPCs than the hepatocytes 24 h after the second dose of ^{14}C -DMH (Table 1). The slower rate of DNA repair and increased amount of metabolic incorporation suggest that non-repair-related DNA synthesis, that is, cell replication, is occurring in the NPCs. This may be due to selective cytotoxicity for the NPCs leading to repopulation.

This present study demonstrates that different cell populations in the intact rat liver possess different capacities for DNA repair. It further shows that in DMH-induced carcinogenesis the target cell population (NPCs) accumulates the promutagenic alkylation product, O^6 -methylguanine, whereas the non-target cell (hepatocyte) does not. It also suggests that the rate of cell division may be higher in the target cells. These data are consistent with the hypothesis that the extent of promutagenic DNA damage, the capacity to repair such damage, as well as the degree of cell replication represent critical events which correlate with carcinogenesis. Previous investigations of organ-specific carcinogenesis^{7,11} have supported this conclusion; however, this is the first demonstration of cell specificity within the target organ.

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L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase

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The release of insulin evoked by nutrients in the pancreatic β -cell is attributed to either the activation of a stereospecific receptor by the nutrient molecule itself or the generation of one or more signal(s) through the intracellular metabolism of the nutrient secretagogue¹. The first of these hypotheses is apparently supported by the fact that nonmetabolized amino acids, especially the L-leucine analogue b(-)-2-amino-bicyclo[2, 2, 1]heptane-2-carboxylic acid (BCH), stimulate insulin release². However, we now report evidence in support of the second hypothesis. We present data consistent with the idea that BCH induces insulin release through the allosteric activation of glutamate dehydrogenase. This is compatible with the fuel hypothesis, which states that the secretory response to nutrient secretagogues depends always on an increase of catabolic fluxes in the islet cells^{3,4}.

The activity of glutamate dehydrogenase in islet homogenates was measured at pH 8.0 and 25 °C in the presence of NH_4^+ 50.0 mM, α -ketoglutarate 7.0 mM and NADH 0.1 mM, as described elsewhere⁵. L-leucine and b(-)-BCH were equally potent in causing allosteric activation of the enzyme (Fig. 1). D-leucine had no effect on enzyme activity (data not shown). The velocity of the reaction catalysed by glutamate dehydrogenase was considerably increased by ADP (0.5 mM). Even in the presence of ADP, L-leucine and b(±)BCH still caused a significant increase in reaction velocity (Fig. 1). L-norvaline and L-isoleucine also caused activation of glutamate dehydrogenase, although to a lesser extent than L-leucine (Table 1). Glycine,

Table 1 Effect of amino acids on glutamate dehydrogenase and insulin release

Amino acid (mM)	Glutamate dehydrogenase (nmol per islet per 60 min)	Insulin release (μ U per islet per 90 min)	
		No glutamine	L-glutamine (10.0 mM)
Nil	2.5 $\pm$ 0.5 (7)	15.4 $\pm$ 1.8 (56)	13.6 $\pm$ 2.2 (40)
L-leucine (10)	9.3 $\pm$ 1.0 (7)*	51.7 $\pm$ 3.0 (54)*	206.7 $\pm$ 18.0 (19)*
b($\pm$)BCH (10–20)	8.9 $\pm$ 1.7 (3)†	24.5 $\pm$ 3.0 (20)‡	185.1 $\pm$ 4.0 (20)*
L-norvaline (20)	6.2 $\pm$ 0.6 (3)†	38.6 $\pm$ 3.0 (29)*	93.1 $\pm$ 8.1 (19)*
L-isoleucine (20)	5.0 $\pm$ 0.9 (5)§	15.7 $\pm$ 1.4 (18)	50.2 $\pm$ 5.5 (28)*
Glycine (10)	3.0 (1)	18.0 $\pm$ 2.7 (19)	25.1 $\pm$ 2.2 (9)
L-serine (10)	3.1 (1)	20.2 $\pm$ 2.5 (10)	19.7 $\pm$ 2.1 (10)
L-valine (20)	2.6 $\pm$ 1.2 (2)	14.8 $\pm$ 2.1 (20)	21.8 $\pm$ 3.9 (20)
L-norleucine (10–20)	2.1 $\pm$ 0.2 (2)	12.7 $\pm$ 1.6 (9)	14.6 $\pm$ 2.2 (17)
L-lysine (20)	2.9 (1)	44.7 $\pm$ 5.0 (30)*	40.3 $\pm$ 7.9 (10)

The activity of glutamate dehydrogenase was measured in islet homogenates. Insulin release was measured in islets incubated in the absence or presence of L-glutamine. Mean values ($\pm$ s.e.m.) are shown together with the number of individual determinations (in parentheses).

* $P < 0.001$; † $P < 0.005$; ‡ $P < 0.02$; § $P < 0.05$. The statistical indices refer either to the differences between basal (first line) and experimental values (first and second columns) or to the glutamine-induced increment in insulin output (third column).

L-serine, L-valine, L-norleucine and L-lysine failed to activate glutamate dehydrogenase. These results are virtually identical to those found in isolated rat liver mitochondria or with purified glutamate dehydrogenase extracted from bovine liver^{6–8}.

The possible relevance of changes in enzyme activity to the secretory behaviour of the β -cell was investigated by examining the influence of L-glutamine (10 mM) on insulin release in islets exposed to various amino acids. This approach was motivated by the following considerations. First, in the islets, L-glutamine is extensively converted to glutamate and, hence, provides much larger amounts of substrate for glutamate dehydrogenase than exogenous glutamate, which is poorly transported into the islet cells⁹. Second, L-glutamine itself fails to affect insulin release by the islets, whether in the absence or presence of D-glucose⁹. Insulin release was measured in groups of eight islets each incubated for 90 min at 37 °C in a bicarbonate-buffered medium¹⁰.

As shown in Table 1, L-glutamine markedly enhanced insulin release provoked by L-leucine or b($\pm$)BCH, augmented

secretion to a lesser extent in the presence of L-norvaline or L-isoleucine, and failed significantly to increase insulin output in the presence of glycine, L-serine, L-valine, L-norleucine or L-lysine. There was a highly significant correlation ($r = 0.9786$; $P < 0.001$) between the magnitude of the glutamine-induced increment in insulin output in the presence of different amino acids and their capacity to activate glutamate dehydrogenase in islet homogenates. In contrast, the magnitude of the glutamine-induced increment in insulin output was not closely related to the capacity of a given amino acid to stimulate secretion in the absence of L-glutamine.

The dose-response relationship for the effect of L-leucine and L-glutamine on insulin release, as illustrated in Fig. 2, indicated that a dramatic augmentation of L-leucine-induced insulin release occurred in the presence of 1.0 mM L-glutamine, at a concentration close to physiological value. The threshold value for L-leucine-induced release (3.0–5.0 mM) was the same in the absence or presence of L-glutamine. However, at higher concentrations of L-leucine, the dose-response relationship was

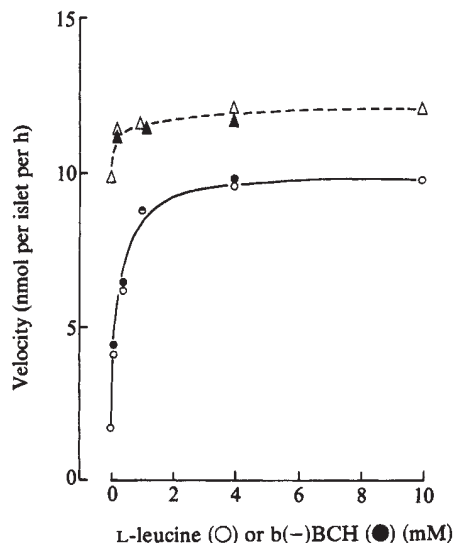


Fig. 1 Effect of L-leucine (open symbols) and b(-)BCH (assumed to represent half of the b($\pm$)BCH concentration effectively used; closed symbols) on the activity of glutamate dehydrogenase measured in islet homogenates in the presence (triangles) or absence (circles) of ADP (0.5 mM). Each value was measured in triplicate.

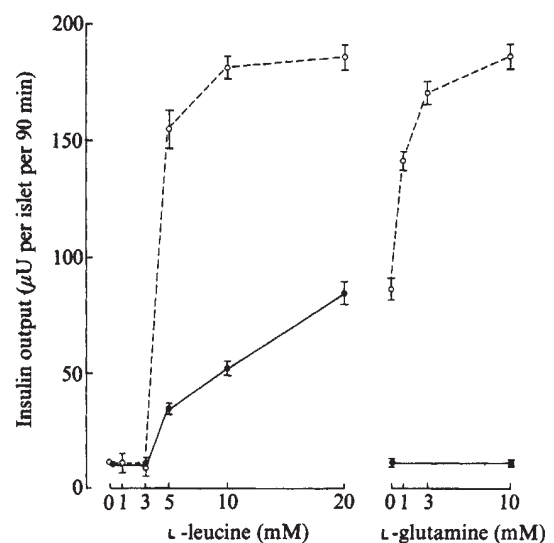


Fig. 2 Mean values ($\pm$ s.e.m.) for insulin release from pancreatic islets refer to 7–136 individual observations. Left panel: effect of increasing concentrations of L-leucine in the absence (solid line) or presence (dotted line) of L-glutamine (10.0 mM). Right panel: effect of increasing concentrations of L-glutamine in the absence (solid line) or presence (dotted line) of L-leucine (20.0 mM).

different in the presence and absence of L-glutamine, respectively. In the presence of L-glutamine, the secretory rate virtually reached a maximal value at a 10 mM concentration of L-leucine. In the absence of L-glutamine, even 20 mM L-leucine was not sufficient to evoke a maximal secretory response, the output of insulin being further increased when the concentration of L-leucine was raised to 40 mM (ref. 11). This difference in dose-response relationship may be explained as follows. In the presence of L-glutamine, the secretory response is largely dependent on the rate of glutamate metabolism, the catabolism of L-leucine itself being severely impaired (data not shown). Hence, a maximal secretory rate will be reached whenever the mitochondrial concentration of L-leucine (or $b(\pm)BCH$) is sufficient to cause full activation of glutamate dehydrogenase. In the absence of L-glutamine, however, the secretory response is largely dependent on the rate of L-leucine catabolism¹¹ and, hence, may require higher concentrations of this amino acid to reach its maximal value. This is not to deny that, in the absence of exogenous L-glutamine, the secretory response to L-leucine depends in part on the rate of endogenous glutamate metabolism, as activated by the branched-chain amino acid. In fact, the extent to which L-leucine, independently of its own catabolism, stimulates insulin release by facilitating the metabolism of glutamate derived from endogenous precursors can be judged from the effect of $b(\pm)BCH$ on insulin release in the absence of exogenous L-glutamine (Table 1 and ref. 12).

Several findings suggest the existence of a causal link between activation of glutamate dehydrogenase and stimulation of insulin release. Thus, in addition to the above-mentioned correlation between enzyme activation by different amino acids and the magnitude of the glutamine-induced increment in insulin output, both the enzyme activation and secretory response¹² are stereospecific for the L-form of leucine. Moreover, detailed biochemical and functional studies performed in intact islets exposed to the combination of L-leucine and L-glutamine indicated that the secretory response to these amino acids is indeed linked to activation of the NAD(P)H-dependent enzyme glutamate dehydrogenase, and is associated with a more reduced state of cytosolic redox couples, abolished by menadione, and triggered by the cellular accumulation of calcium and subsequent activation of the β -cell microtubular-microfilamentous effector system (data not shown). These findings emphasize the essential role of reducing equivalents in the coupling between metabolic and cationic events in the process of nutrient-induced insulin release³.

In conclusion, the present data indicate that the capacity of a nonmetabolized amino acid to augment O_2 consumption by the islets¹³, to increase their content in NAD(P)H (ref. 14), to provoke a phosphate flush¹⁵ and to stimulate insulin release² is compatible with the fuel hypothesis for insulin release³.

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Radioimmune, radiobinding and HPLC analysis of 2-5A and related oligonucleotides from intact cells

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The enzyme (2-5A synthetase) which synthesizes $ppp(A2'p)_nA$ where $n = 2$ to 4 (collectively referred to as 2-5A)¹ is widely distributed in a variety of cells and tissues in amounts which increase in response to interferon²⁻⁵ and vary with growth and hormone status⁶. 2-5A activates a nuclease which inhibits protein synthesis⁷⁻¹⁰. The non-phosphorylated 'core' of 2-5A ($(A2'p)_nA$, $n = 2$ to 4) can inhibit DNA synthesis and cell growth^{11,12}. Here we describe convenient and sensitive radioimmune (RI) and radiobinding (RB) assays for core and 2-5A. In combination with more satisfactory high performance liquid chromatography (HPLC) methods using reverse-phase C18 columns, these assays have been used to detect core and 2-5A in crude extracts from interferon-treated cells. The novel 2-5A synthetase products $NAD2'p5'A2'p5'A$ and $A5'p5'A2'p5'A2'p5'A$ (ref. 13), which can also be detected using the RB assay, were not found in significant amounts. The natural occurrence of core has not been described previously.

The RI and RB assays are based on the high affinities of core for antibody and of 2-5A for the 2-5A-dependent nuclease^{9,10} respectively. In both cases the amount of ³²P-labelled core or 2-5A bound to antibody or nuclease is determined by its retention on nitrocellulose filters. The concentration of unknown samples is measured by the ability of suitable dilutions to

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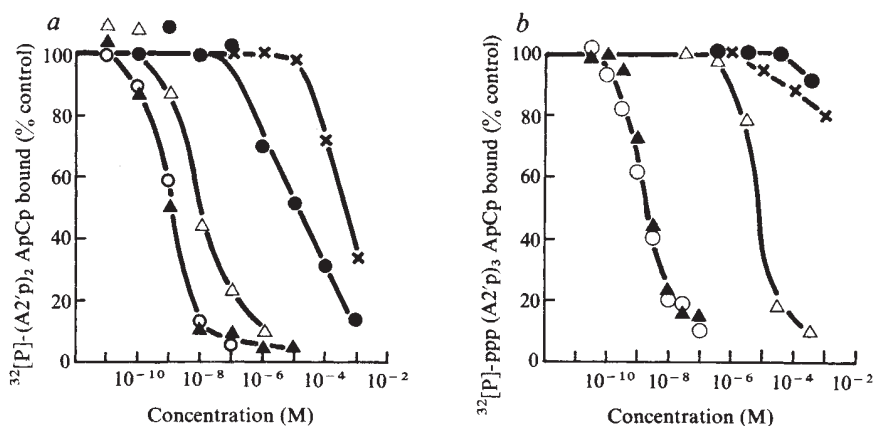
Table 1 Specificity of the radioimmune and radiobinding assays

Compound	Concentration for 50% displacement of probe*
a, Radioimmune assay	
$(A2'p)_2A$ and $(A2'p)_3A$	1 nM
$p(A2'p)_2A$	5 nM
$pp(A2'p)_2A$	100 nM
$ppp(A2'p)_2A$	500 nM
2'- and 3'-AMP	5 μ M
5'-AMP	10 μ M
$(A3'p)_2A$ and $(A3'p)_3A$	10 μ M
$A3'p5'A$	50 μ M
Adenosine	500 μ M
ATP, CTP, pCp, 2'-, 3'- and 5'-	>1 mM
CMP	
b, Radiobinding assay	
$ppp(A2'p)_nA$, ($n = 2$ to 4)	1 nM
$pp(A2'p)_2A$	1 nM
$p(A2'p)_2A$	7 nM
$(A2'p)_2A$	300 nM
$(A3'p)_2A$ and $(A3'p)_3A$	>0.3 mM
2'-, 3'-, and 5'-AMP, ADP, ATP	>1 mM
2'-, 3'- and 5'-CMP, pCp	>1 mM

The assays were carried out as for Fig. 1. Concentration curves were constructed for each of the compounds tested for which the 50% displacement values given were obtained. Essentially identical results to those shown in Fig. 1a and Table 1b were obtained using the RI assay with ³H-(A2'p)A as probe except that the assay was approximately 20-fold less sensitive, reflecting the lower specific activity of this material.

* In the RI and RB assays the radioactive probes were $(A2'p)_2A$ -[³²P]pCp and $ppp(A2'p)_3A$ -[³²P]pCp respectively.

Fig. 1 Radioimmune (RI) and radiobinding (RB) assays for A2'p5'A2'p5'A, pppA2'p5'A2'p5'A and related oligonucleotides. *a*, The RI assay. Inhibition of the binding of (A2'p)₂A-[³²P]pCp to (A2'p)₂A antiserum by different concentrations (abscissa) of unlabelled (A2'p)₂A, (○); (A2'p)₃A, (▲); A2'p5'A, (△); (A3'p)₂A, (●) and adenosine (×). *b*, The RB assay. Inhibition of the binding of ppp(A2'p)₃A-[³²P]pCp to the 2-5A-dependent nuclease by different concentrations (abscissa) of unlabelled ppp(A2'p)₂A, (○); ppp(A2'p)₃A, (▲); pppA2'p5'A, (△); (A3'p)₂A, (●) and adenosine, (×). The RI assay (55 μl) contained 3,000 c.p.m. of (A2'p)₂A-[³²P]pCp (1–3 × 10⁶ Ci mol⁻¹), 15 μl of the sample being tested and 25 μl of a 1:2,000 dilution of antiserum. All dilutions were in Tris-HCl-buffered saline pH 7.6 (TBS); 0.1% gelatin. Incubation was for 1 h at 30 °C. Samples (40 μl) were applied to nitrocellulose filter discs (2.5 cm diameter, 0.45 μm pore size; Sartorius) which were washed in three changes of TBS, dried and counted in a toluene-based scintillant. The RB assays (20 μl) contained 3,000 c.p.m. of ppp(A2'p)₃A-[³²P]pCp (1–3 × 10⁶ Ci mol⁻¹), 2 μl of competitor, 10 μl of a post-mitochondrial supernatant fraction¹⁹ from Ehrlich ascites tumour cells as the source of the 2-5A-dependent nuclease and 8 μl of 20 mM Tris-HCl pH 7.6, 8.5 mM magnesium acetate, 1 mM ATP and 5% glycerol (v/v). Incubation was for 60 min at 0 °C. Samples (19 μl) were applied to pre-wet nitrocellulose filters as above which were washed in TBS, dried and counted. In both assays the conditions were chosen to give 30 to 50% binding of the radioactive probe in the absence of competitor. This corresponds to the 100% point of the ordinate. The (A2'p)₂A antiserum was prepared in rabbits with (A2'p)₂A bound to bovine serum albumin as antigen^{20–22}. The 5'-[³²P]pCp labelled derivatives were synthesized with T₄ RNA ligase and purified by HPLC (R.H.S., unpublished results and ref. 22).



displace 50% of the bound ³²P-labelled derivative in comparison with the concentration of pure core or 2-5A required to produce a similar displacement. (A similar nitrocellulose filter procedure, but for the detection of the nuclease, was used by Slattery *et al.*¹⁰.)

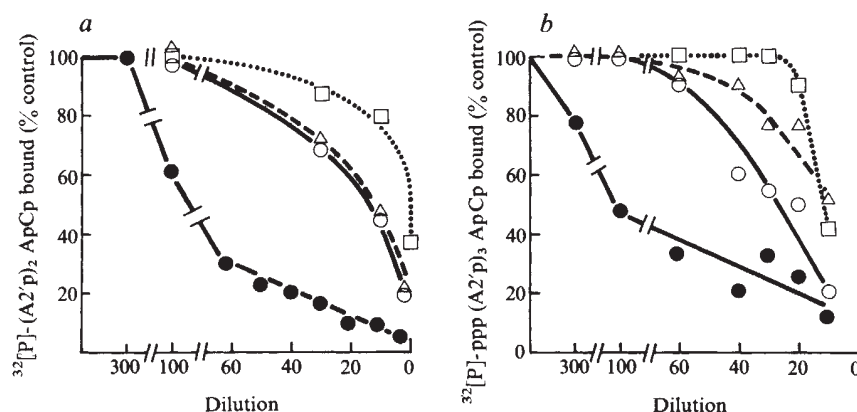
The difficulty in developing suitable assays has been in the production of 2-5A of sufficiently high specific activity (to detect ≤10 μl of nanomolar 2-5A). In our experience, very poor yields of 2-5A are obtained with the 2-5A synthetase when substrate (ATP) concentrations of ≤100 μM are used, making the production of 2-5A of specific activity ≥10,000 Ci per mole of nucleotide costly and impractical. Accordingly, here we have utilized T₄ RNA ligase^{14,15} to label preformed (A2'p)₂A or ppp(A2'p)_nA (*n* = 2 or 3) with [5'-³²P]pCp to specific activities of 2–3 × 10⁶ Ci per mole of oligonucleotide.

The RI assay shows high but incomplete specificity for the 2'-5' linkage (Fig. 1*a*, Table 1*a*). Fifty per cent displacement of the radioactive probe is observed with nM (A2'p)₂A or (A2'p)₃A (○, ▲, Fig. 1*a*) and 10 nM A2'p5'A (△, Fig. 1*a*). The concen-

tration at which other potentially cross-reacting materials displace 50% of the probe are given in Table 1*a*. The RI assay provides a sensitive method for the detection of core but without prior fractionation it cannot be used to measure core in the presence of higher concentrations of 2-5A (Table 1*a*). In addition, problems will arise in the assay of crude material of low titre if it contains sufficient AMP, ADP and ATP to yield ≥500 μM adenosine on dilution into the assay after digestion with bacterial alkaline phosphatase (BAP), or ≥10 μM 3'-5' (Ap)_nA (*n* = 2 or 3) (Table 1*a*). It is possible that other 3'-5'-linked oligonucleotides may also cross-react.

The RB assay shows even greater specificity for 2-5A (Fig. 1*b* and Table 1*b*). The 5' triphosphorylated (○, Fig. 1*b*) and diphosphorylated (Table 1*b*) trimer, the tetramer (▲, Fig. 1*b*) and pentamer (Table 1*b*), all displace 50% of the bound probe at nanomolar concentrations. In fact it shows almost as great a specificity as the 'biological' assays for 2-5A. It falls short of this in detecting relatively low concentrations of the trimer 5'-monophosphate and higher concentrations of core (Table 1*b*),

Fig. 2 Radioimmune (*a*) and radiobinding (*b*) assays for the natural occurrence of 2-5A in extracts from interferon-treated, EMC virus-infected (●); interferon-treated (○); EMC virus-infected (△) and control (□) mouse L-cells. The assays were carried out as described in Fig. 1 legend. The 100% values correspond to 1,300 c.p.m. representing the 30 to 50% of the radioactive probes bound in the absence of competitor. Mouse L-cells (0.75 × 10⁹ cells) were treated for 17 h at 37 °C with 400 reference (20 effective) units per ml of partially purified (5 × 10⁷ reference units per mg of protein) mouse interferon, infected with 20 plaque-forming units (PFU) per cell of EMC virus and incubated for 4 h at 37 °C. Interferon-treated cells and control cells with or without EMC infection were incubated in parallel. Cells were collected, washed and the packed cells (2.5 ml) lysed into an equal volume of 0.5% NP40 in 10 mM HEPES buffer pH 7.5; 90 mM KCl; 1.5 mM magnesium acetate at 0 °C. Trichloroacetic acid (50%) was added immediately to 5% and the mixture centrifuged at 2,000g for 20 min. The supernatant solutions were extracted 10 times with water-saturated ether, lyophilized and resuspended in 500 μl of 10 mM HEPES buffer pH 7.5 (the pH was adjusted to 7 with NH₄OH). These extracts were assayed with (radioimmune) or without (radiobinding) prior BAP digestion (40 units of enzyme (Sigma) per ml overnight at 30 °C). Control experiments in which radioactive 2-5A or core (200 nM) were added at the time of cell lysis showed recovery through the remainder of the procedures to be ≥50%.



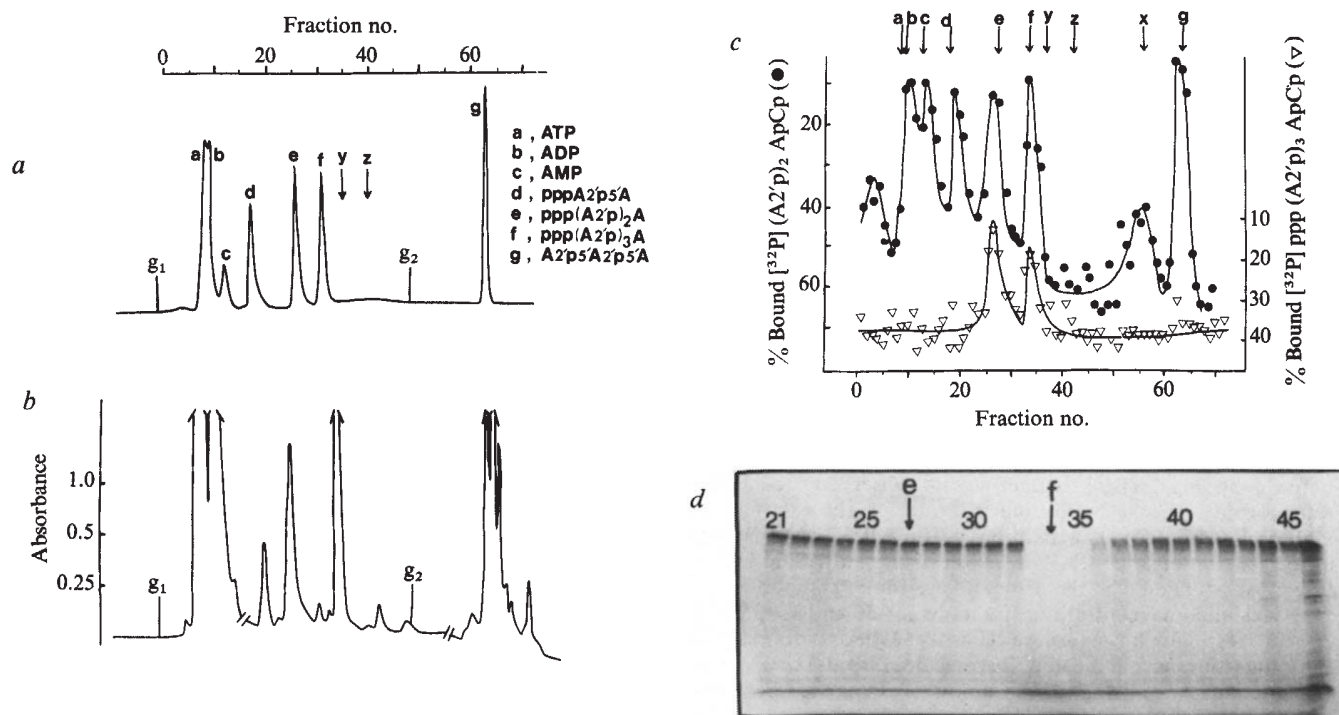


Fig. 3 HPLC analysis of 2-5A from interferon-treated EMC virus-infected L-cells. *a*, A mixture of standards containing ATP, ADP, AMP and the dimer, trimer, tetramer and core of 2-5A (a-g). The positions y and z at which $A_5'p_45'A_2'p_5'A_2'p_5'A$ and $NAD_2'p_5'A_2'p_5'A$, respectively, eluted in parallel runs are also indicated on the figure. *b-d*, Cell extract (150 μ l) from 0.75 ml of packed cells. *b*, Absorbance profile: absorbance was measured at 256 nm and at fraction 17 and fraction 57 the full-scale deflection (2.56) was reduced twofold (to 1.28) and 32-fold (to 0.08). *c*, RB assay: column fractions (5 μ l) were assayed for their ability to displace $ppp(A_2'p)_3A-^{32}P]pCp$ and RI assay: column fractions (15 μ l) were assayed after BAP digestion (as in Fig. 2) for their ability to displace $(A_2'p)_2Ap-^{32}P]pCp$. The amounts of trimer, tetramer and core detected in the RI assay were unaffected by prior digestion of the fractions with ribonuclease T₂. This excludes any possibility that the titres obtained reflect the presence of as yet unidentified cross-reacting 3'-5'-linked material. *d*, Nuclease assay¹⁷: fractions (25-46 and 58-70) (1 μ l) were analysed for their ability to activate a nuclease and degrade ^{32}P -RNA in a reticulocyte lysate system¹⁷. Activation of the nuclease by: C, no additions; S1-6, 0.02, 0.06, 0.2, 0.6, 2.0 and 6.0 nM 2-5A tetramer standard; D1-5, dilutions, 1,250-, 375-, 125-, 37.5- and 12.5-fold of the interferon-treated, EMC virus-infected L-cell extract. The interferon treatment and EMC infection of the L-cells and preparation of the trichloroacetic acid extracts were as described in Fig. 2 legend. The HPLC analysis was on a μ Bondapak C18 column (Waters Associates) run in 50 mM ammonium phosphate, pH 7.0 at 1 ml min⁻¹ with a 25-min linear gradient of 50:50 methanol:water applied at g_1 to reach a final of 20% of the total flow by g_2 where the methanol:water was increased to 50% in a 5-min gradient. This second gradient does not separate the 2'-5'-linked oligomers. Good separation of these can be achieved if a shallower gradient is applied at g_2 . The individual 2'-5' and 3'-5'-linked core from the corresponding 3'-5'-linked core components can be resolved in a gradient of methanol in 4 mM potassium phosphate pH 6.5. (It is advisable in the routine analysis of cell extracts that a dry packed pre-column should be inserted to protect the main column.)

both of which are without effect in the activation of the 2-5A-dependent nuclease or on cell-free protein synthesis. All of the other potentially cross-reacting materials tested were essentially without effect (Fig. 1*b*, Table 1*b*).

The specificity is sufficient for the RB assays to be performed with crude cell extracts as the source of the 2-5A-dependent nuclease as binding protein. The results presented here were obtained with post-mitochondrial supernatant fractions from Ehrlich ascites tumour (EAT) cells and very similar results have been obtained with rabbit reticulocyte lysates. It is possible that crude extracts of this type contain other proteins which contribute to the specific binding of 2-5A. However, the results obtained after partial purification of the nuclease and in experiments involving the analysis of radioactive 2-5A covalently bound to protein in such extracts favour the nuclease as the major component involved (D.H.W., C.S.G., R.H.S. and I.M.K., unpublished results). Less extensive studies with similar extracts from HeLa and L-cells suggest that they (and probably any extract containing significant amounts of the 2-5A-depend-

ent nuclease) can also be used in this type of assay. Care should, however, be taken to monitor for significant degradation of 2-5A by such extracts in the development of suitable assays^{9,16}.

Despite the relatively high specificity of the RI and RB assays, the exclusion of cross-reacting material is potentially a problem in the assay of crude extracts. Nevertheless, in agreement with our previous results using 'biological' assays¹⁷, both the RI (after BAP) and RB assays (Fig. 2*a* and *b*, respectively) indicated relatively high concentrations (~100 nM in trimer equivalents) of 2-5A±core in a trichloroacetic acid-soluble extract from interferon-treated, EMC virus-infected mouse L-cells (●) and only much lower concentrations in corresponding extracts from interferon-treated (○), or EMC-infected (Δ), or control (□) cells. That the majority of the material registering in these assays was indeed 2-5A or core was confirmed by HPLC.

The results presented in Fig. 3*b-d* are of an HPLC analysis of an acid-soluble extract from interferon-treated, EMC virus-infected mouse L-cells. The eluates were first assayed by their ability to activate a nuclease in reticulocyte lysates (Fig. 3*d*)¹⁷. A

Table 2 Levels of 2-5A and related oligonucleotides in interferon-treated and control mouse L-cells with or without EMC virus infection

Pretreatment of cells	Concentration in the intact cell (nM)				
	2-5A		Core	NAD2'p5'A2'p5'A	Ap ₄ A2'p5'A2'p5'A
	Trimer	Tetramer			
Interferon and EMC infection	100	100	150	<5	<5
Interferon	30	30	20	<5	<5
EMC infection	10	10	<2.5*	<5	<5
Control	<2.5	<2.5	<2.5	<5	<5

The concentrations given are based on the packed cell volumes from which the extracts were made. They are calculated from the concentrations recovered after HPLC analysis carried out as for Fig. 3. No allowance has been made for losses during the extraction and processing of the samples. Control experiments in which 2-5A and the related oligonucleotides (50 nM) were added at the time of cell lysis indicated that the recovery of each component was $\geq 50\%$.

* In one experiment in which two- to fourfold higher concentrations of 2-5A were found in the interferon-treated cell extracts (with or without EMC infection) a low level of core (≤ 8 nM) was detected in the extract from EMC-infected cells.

single peak was obtained corresponding to the tetramer (fractions 33–36, Fig. 3d). The trimer was not observed because the reticulocyte nuclease is unusual in requiring the tetramer or higher oligomers for its activation¹⁷. The RB (∇) and RI ($\bullet$) assays (the latter after BAP digestion of the individual HPLC fractions) both detected the trimer and tetramer components of 2-5A at similar concentrations (fractions 25–30 and 33–35, Fig. 3c). Interestingly, however, the RI assay (with or without prior BAP digestion) also indicated the presence of pre-existing core ($\bullet$, fractions 62–64, Fig. 3c) in amounts similar to those observed for the phosphorylated components. Further HPLC analysis of this material using a gradient of methanol in 4 mM potassium phosphate pH 6.5 showed it to consist largely of the trimer core ((A2'p)₂A). There was no evidence for significant breakdown of 2-5A to core in control experiments in which low concentrations (<100 nM) of 2-5A were added at the time of cell lysis. This, together with the results of experiments in which the time taken to wash and disrupt the cells was deliberately varied, suggests that the core is not an artefact.

The RI assay also detected dimer ($\bullet$, fractions 19–21, Fig. 3c) and cross-reacting material in fractions 1–15 which is probably adenosine produced by the BAP digestion of ATP, ADP and AMP. The concentration of this material is high compared with that in the corresponding control extract (not shown). This emphasizes the necessity for HPLC or alternative additional analysis of material detected by the RI assay in crude extracts in order to establish its identity.

The nature of peak X (Fig. 3c) is not known. It does not seem to be 2-5A joined to NAD¹³. The latter has been synthesized *in vitro* from NAD and ATP with the 2-5A synthetase bound to poly(I)-poly(C)-paper in conditions similar to those used for 2-5A^{2,6,13}. The products NAD2'p5'A and NAD2'p5'A2'A were purified by chromatography (on DEAE-cellulose) and the structure of the latter was confirmed by proton NMR spectroscopy. On HPLC analysis the product NAD2'p5'A2'p5'A (Z, Fig. 3a) eluted after NAD. It can be detected at nanomolar concentrations using the RB assay. With the extract from interferon-treated, EMC-infected cells, only very low levels of material (equivalent to ≤ 5 nM of putative NAD2'p5'A2'p5'A) were detected in this region of the gradient (Fig. 3c). Control experiments in which NAD2'p5'A2'p5'A at 50 nM was added at the time of cell lysis have shown 75% recoveries. Similar results have been obtained in a less extensive investigation into the possible presence of A5'p₄A2'p5'A2'p5'A (Y, Fig. 3a, c). It is unlikely, therefore, that either NAD or A5'p₄A (ref. 18) modified by the addition of 2-5A occurs naturally in these cells at concentrations greater than 5 nM.

Similar HPLC analysis of extracts from interferon-treated L-cells and from EMC virus-infected L-cells, have shown that they contain 2-5A and (in the case of the interferon-treated cells at least) core at lower concentrations (Table 2). It is not yet clear whether very low levels (3 nM in the intact cell) of core and 2-5A are also present in control extracts (Fig. 2 and Table 2).

The results presented establish the validity of the RI and RB assays to detect core and 2-5A. They are now the methods of

choice for the assay of preformed core and 2-5A being much more convenient than the 'biological' assays. In addition the assays are suitable for the routine screening of cells and tissues for 2-5A. The identity of any putative 2-5A components or 2-5A-related oligonucleotides detected in such material must, however, be confirmed by further HPLC (or alternative) analysis.

The natural occurrence of 2-5A and 'core' in interferon-treated cells, although at lower concentrations than in interferon-treated EMC virus-infected cells (Table 2), emphasizes the possible involvement of the 2-5A system in the cell growth inhibitory activity of interferon. The presence of low levels of 2-5A in virus-infected cells may reflect the low levels of synthetase known to be present in the absence of interferon treatment and its activation by viral double-stranded RNA⁶.

The natural occurrence of substantial amounts of core (Table 2) is of considerable interest and emphasizes that in the analysis of the 2-5A system it is not adequate to measure only 2-5A. The amount of core seems to be independent of the concentration of 2-5A and it may, therefore, play a separate role in the inhibition of DNA synthesis and cell growth.

We thank B. F. Erlanger and S. Zakarian for advice in setting up the radioimmune assays, B. F. Erlanger for antisera to 3'-5'-linked ApApA used in preliminary studies, and S. Jones and C. B. Reese for chemically synthesized 2-5A trimer, 5'-mono-, di- and triphosphates. M.K. was the recipient of a grant from the government of Ghana and D.H.W. of an EMBO fellowship.

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MATTERS ARISING

Origin of mammal-like reptiles

KEMP¹ has recently hypothesized that "the synapsid [mammal-like reptile] skull evolved from a limnoscelid-like skull rather than from that of a romeriid, and that the temporal fenestra of the mammal-like reptiles evolved directly from the remnant of the crossopterygian hinge line". This disagrees with the widely accepted theory that the Protorothyrididae (Romeriidae), a group of small reptiles with unfenestrated skull roofs, include the ancestors or near-ancestors of the Pelycosauria, the earliest synapsid or mammal-like reptiles²⁻⁴. Both Kemp¹ and Reisz^{5,6} have recognized that the protorothyridid skull is advanced in some cranial features compared with pelycosaurs and, hence, could not be ancestral to the primitive pattern of early synapsids. Kemp's hypothesis replacing a protorothyridid ancestor with a "limnoscelid-like" ancestor also seems invalid.

Kemp uses the common occurrence of a large supratemporal that contacts the postorbital, and a large tabular that contacts the paraoccipital process of the opisthotic, as the basis of his hypothesis. These, however, are primitive tetrapod characters present in virtually all primitive amphibians and reptiles. Thus they have no phylogenetic significance in the context of synapsid origins. The proper methodology for developing a theory of phylogenetic relationships of any group of organisms is to study the distribution pattern of morphological characters among a whole range of generally similar organisms so that a determination of whether a particular character is primitive or derived may be made (only derived characters are of any significance when trying to deduce phylogenetic relationships). Because Kemp has failed to do this, he has been badly misled by phylogenetically insignificant primitive characters.

The thesis that the synapsid temporal fenestrae evolved directly from a remnant of the crossopterygian hinge-line between the skull roof and cheek is untenable. There is no evidence that any true cotylosaur, including seymouriamorphs or diadectomorphs (limnoscelids belong to the latter group⁷) or primitive reptiles, ever had a cardinal line of weakness (or hinge) between the skull roof and cheek in the living animals. The presence of such a feature in the above animals is an old misconception based on the fact that post-depositional crushing tends to concentrate forces along the sharp angle between the skull roof and cheek leading to breakage of the bone or suture. The only known skull of *Limnoscelis* is compressed dorsolaterally so that the bone has broken in this region to give the impression of a

hinge, or line of weakness. As Heaton⁸ has noted, this often happened in captorhinomorphs as well, where there was a solid wide suture between the skull roof and cheek. The only known specimen of *Romeriscus*, a possible limnoscelid on which Kemp placed much emphasis, is so poorly and incompletely preserved that it cannot be used as the basis of a major hypothesis.⁹

Hypotheses on the origin of the Synapsida based on the development of the temporal fenestrae¹⁰ are inappropriate because these are not restricted to them (lateral temporal fenestrae are present in mesosaurs, bolosaurs, diapsids and some millerosaurs) and because fenestration is only one aspect of the development of the synapsid skull. Any hypothesis of the origin of the synapsid reptiles should be linked to the structural innovations seen in the braincase, occiput, adductor chamber, mandibles and dentition as a whole, because these changes gave rise to a morphological complex that is unique among higher vertebrates.

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KEMP REPLIES—Reisz and Heaton's criticism of the use of primitive characters to demonstrate phylogenetic relationships ignores my specific disclaimer that I was proposing any particular relationship between limnoscelids and synapsids. My object was simply to suggest that the character-state of the temporal region of the skull of the direct ancestor of synapsids resembled that retained in the limnoscelids, for example, and not that of romeriid or protorothyridid reptiles. Of course any hypothesis about the phylogenetic relationships of synapsids to particular tetrapod groups would require a comprehensive study of the distribution of derived characters of the whole skeleton. It was for this reason that I

carefully avoided any taxonomic implications at all, and indeed the possibility of a primary sister-group relationship between romeriids and synapsids is nowhere excluded.

The exact nature of the connection between the skull table and cheek of *Limnoscelis* is difficult to determine from a single, damaged skull. Even if the 'hinge-line' is merely the consequence of distortion, as Reisz and Heaton now claim, the similar manner of breakage of the two sides of the skull, between the table and the cheek, suggests nevertheless that this line was weak. So also does the fact that the suture between the post-orbital and squamosal is continued posteriorly in a straight line by the suture between the supratemporal and squamosal to the hind margin of the skull. My contention that the synapsid temporal fenestra appeared within this suture line remains unaffected, even if the contact between the respective bones was rather firmer than supposed. There would still have been a thin layer of connective tissue between the bones, as in the case of any suture, which could have expanded, eventually forming the temporal aponeurosis.

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Growth rings in dinosaur teeth

THERE are several difficulties with Johnston's interpretation of the growth rings he has observed in dinosaur teeth¹. The first problem is whether the rings are annual. Such growth rings are found in living and fossil ectothermic vertebrates, but in the bones, not the teeth, which are replaced frequently in most non-mammalian vertebrates². The teeth of crocodiles are replaced at intervals of 8-16 months³, making it impossible for more than two annual rings to occur within any given tooth. There is no evidence that replacement ceases during adult life, nor that the rate was substantially different in fossil crocodilians. Data on the manner and rate of tooth replacement for dinosaurs are limited, although evidence suggests multiple replacement and rapid turnover².

Dinosaurs and crocodiles would be expected to share some similarities in hard-tissue deposition and to differ from mammals, for the ancestors of archosaurs and mammals were separated perhaps as

far back as latest Carboniferous⁴. Johnston ignores conclusions based on extensive studies of fossil reptile bones⁵ which show that dinosaur bone resembles more that of large living endotherms than ectotherms. He argues that, because crocodiles are ectothermic and their teeth are similar to those of dinosaurs, then dinosaurs also must have been ectothermic. But similar teeth are also found in Cretaceous birds^{6,7}, implying either that they were ectothermic or that dental growth rings are not a sufficient indicator of overall physiology.

The precise connection between growth rings and thermal physiology is unknown, and it is likely that a complex set of phenomena is involved. Mammalian growth rings appear in constant as well as seasonal environments⁸; neither temperature nor moisture are proven causal factors. Endo/ectothermy is not a sharp dichotomy, but a continuous spectrum, as is homeo/poikilothermy⁹. Calcium stress may be due to seasonal, dietary, reproductive, structural, phylogenetic or other poorly understood influences; the pitfall of 'one-factor' ecology is that no one factor, internal or external, is likely to explain all observed metabolic responses. Mammals and birds have used various strategies to solve problems in energetics; the diversity of dinosaurs suggests that many physiological solutions may also have been available to them¹⁰. It seems imprudent to rule out other factors contributing to the growth of hard tissues merely because they cannot be accounted for.

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CONTRARY to Johnston's interpretation, the skeletal annuli he observed in dinosaur teeth¹ need not imply any seasonal temperature fluctuation but may simply be a result of seasonal variation in food resources created by seasonal rainfall patterns. Spinge² has shown that two distinct annual cementum lines are

formed in the teeth of African buffalo (*Syncerus caffer*) from regions with bimodal precipitation whereas one exceptionally distinct annulation forms each year in buffalo from southern Tanzania where rainfall patterns are unimodally seasonal. Also, desert bighorn sheep (*Ovis canadensis*) possess annuli in both cementum and dentine resulting from a cessation of growth during dry summer months³. These observations of seasonal dental annuli formation in endotherms counter Johnston's arguments for ectothermy in dinosaurs.

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JOHNSTON¹ claims that 'growth zones' observable in dinosaur teeth give tooth age in years, and suggest that dinosaurs were crocodile-like ectotherms. Individual ages in many mammalian species can be determined by counting various types of growth rings, including those in dentine. But if the results are not checked against individuals of known age²⁻⁴, serious errors can be made. One possibility is that annual growth lines are confused with the contour lines of Owen. Johnston says that there are "two main types of rings" in dinosaur teeth, with the implication that annual rings are easily distinguished. Studies on mammalian teeth suggest that true annual rings are apparently formed in the same way as, and are morphologically similar to, contour lines of Owen⁵.

In the absence of studies on a living relative or other acceptable analogue, it is easy in growth ring studies for hypothesis to bias observations. We suspect that Peabody's paper⁵ cited by Johnston gives an example of this. Peabody recognized as many as four annual growth zones in some teeth of the small Lower Permian reptile *Captorhinus*. Although he did not discuss tooth replacement, a common opinion among vertebrate palaeontologists at the time was that the teeth of *Captorhinus* were not replaced. It would have been reasonable to expect individual teeth to persist for 4 yr. However, it is now known that all the teeth of *Captorhinus* were being actively replaced⁷. It is unlikely that a single tooth in such a small animal would persist for 4 or more years; Peabody's 'annual growth zones' probably reflect a less than annual periodicity if they had a regular periodicity at all.

Because dinosaurs are extinct Johnston's 'annual growth zones' cannot be verified, and his counts can at best be established only as more or less reason-

able by study of living animals. The crocodilians are the closest living toothed relatives of dinosaurs, but there is no substantial evidence as to how long their teeth last or whether they can be aged by counting growth zones. Although Edmund⁸ found a life span of slightly more than 2 yr in *Alligator* teeth, his specimens were very small and do not provide a good parallel with dinosaurs. Neill⁹ states that old alligators cease replacement and may become nearly toothless. Even if this is confirmed, it tells us nothing about the age of the remaining teeth or the number of growth zones they may contain. In any case Johnston's counts of eight annual rings in each of two tyrannosaur teeth seems rather high (though not impossible) in view of the fact that tyrannosaurs, as well as the other dinosaurs he cites, show active tooth replacement^{6,10} which seems to have continued throughout life as we know of no evidence to the contrary.

Johnston notes that de Ricqlès' histological surveys^{11,12} did not reveal seasonal growth rings in dinosaur bone. However, histology did not simply produce negative evidence. de Ricqlès argued cogently, on the basis of long-bone histology, that dinosaurs were probably relatively endothermic. Absence of cyclical growth lines in dinosaur periosteal bone was attributed to the rapid growth characteristic of endotherms. The growth zones observed in dinosaur teeth thus should not be considered in isolation; their interpretation must be consistent with a total histological picture that includes the rest of the skeleton.

Johnston's suggestion of crocodile-like endothermy in dinosaurs at first appears to contradict de Ricqlès, but the latter noted that animals may be neither fully endothermic nor fully ectothermic and that, although dinosaurs were relatively endothermic, they probably had their own peculiarities in thermal physiology. It is impossible to correlate environmental and/or endogenous factors with the formation of growth zones in dinosaur teeth. But even if Johnston is right in calling these growth rings seasonal, their existence simply suggests that de Ricqlès was right in supposing that dinosaurs were not completely comparable to large mammals in thermal physiology. It does not imply, in view of the total histological evidence that dinosaurs were crocodile-like ectotherms.

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JOHNSTON REPLIES—Meinke *et al.* acknowledge that annual layers occur in bone of fossil (and living) ectotherms but deny that the similar rings which occur in the dentine of those animals are annual or even extrinsically induced by the change in seasons. Irrespective of anything else, however, this view is illogical: dentine is itself simply acellular bone. Furthermore, in Recent temperate and arctic terrestrial mammals^{1,2} (marine mammals, cited by Meinke *et al.*, are ecologically inappropriate as analogues of terrestrial crocodiles and dinosaurs): (1) the most conspicuous dentine bands form simultaneously with annual layers in periosteal bone, and (2) dentine has been shown to be a sensitive indicator of annual, seasonally induced disruptions of growth. Meinke *et al.* give no evidence that the dentine in ectotherms (living or extinct) behaves differently.

Boyce, noting that severe seasonal contrasts can produce rings in the dentine of certain terrestrial mammals, overlooks my citation of the same phenomenon³; and Boyce provides no evidence that Alberta dinosaurs lived in seasonally xeric conditions comparable to those of desert bighorn sheep. Instead, palaeontological evidence^{4,5} indicates that the western interior of North America was a humid, warm temperate to subtropical environment without extreme seasonal fluctuation of precipitation and temperature during Late Cretaceous time.

Bolt and DeMar cite possible uncertainty in distinguishing the boundaries of annual layers in dentine (and bone) owing to the production of accessory bands (accentuated contour lines of Owen); however, these difficulties no more detract from the reality of seasonal disruptions in dentine deposition than does the occurrence of false annuli negate the phenomenon of true annuli in fish scales. Furthermore, annual layers in the calcified tissues of Recent terrestrial mammals (and by extrapolation, crocodiles and dinosaurs) can usually be distinguished: they are more pronounced and regularly spaced than are accessory bands^{1,2}.

Meinke *et al.* err in claiming the impossibility of finding more than two annual rings within a crocodile tooth: the number of annual layers in crocodile dentine gives the age of the tooth (minimum age if the pulp cavity is occluded), not just its functional age. In one 100-yr-old crocodile⁶, each tooth probably has been replaced about 50 times—an average of about once every 2 yr. In the Nile crocodile, two or three replacing teeth can be lodged beneath a functional one, and as many as six replacing teeth have been recorded at a single locus⁶. Thus, in a mature individual, a tooth could easily reside in the jaw for 4 yr, two before eruption and two after (longer if more than one successional tooth occurs below a given functional tooth), resulting in the accumulation of four annual layers in the dentine.

The number of major bands in dinosaur teeth is consistent with probable replacement rates: a maxilla of *Antrodemus* sp. (Princeton University Museum No. 16554–9), for example, exhibits three generations of teeth⁷, and if the average tooth replacement interval in mature *Antrodemus* was 2 yr (not unreasonable by analogy with Recent crocodiles), each tooth must have been held for 6 yr (4 yr before eruption and two while functional), yielding six annual layers in the dentine. If the replacement interval ranged from 1 to 3 yr during the life of an individual, the total life of a given tooth could be from 3 to 9 yr depending on the age of the individual. Although absolute replacement rates are not known for dinosaurs, relative rates can sometimes be inferred: hadrosaur and ceratopsian teeth, which are stacked for evident rapid replacement in a specialized grinding mill, generally have fewer rings than do carnosaur teeth, the function of which is not dependent on occlusal wear of crowns.

Bolt and DeMar's model⁸ of tooth replacement in *Captorhinus* describes the pattern of replacement but says nothing of rate. Small size alone is not necessarily indicative of rapid replacement: *Sphenodon* (Rhynchocephalia) and certain acrodont lizards are *Captorhinus*-sized or smaller, yet the lateral teeth wear heavily with age and are probably not replaced at all in mature individuals⁹. There is no histological evidence to support Bolt and DeMar's model of rapid replacement (in fact, heavy wear on many specimens of *Captorhinus*^{8,10} suggests relatively slow replacement) nor can the model account for the occurrence of the tallest teeth at or near the centre of the rows of teeth, a definitive feature of *Captorhinus* multiple-rowed dentitions¹¹ (R. C. Fox, personal communication). Consequently, Peabody's interpretation¹² of growth zones in *Captorhinus* teeth remains a reasonable one.

Mesozoic toothed birds do not show dinosaur or crocodile-like zones in their

teeth (*contra* Meinke *et al.*) (see Yale Peabody Museum specimens 1206, 1474, uncatalogued thin-sections, all teeth of *Hesperornis*, YPM 1728, teeth of *Ichthyornis*); possibly Meinke *et al.* observed ringed teeth in small mosasaur (Lepidosauria) jaws (having lacertilian rows of nutritive foramina), for dentigerous fragments of two such jaws are in the Yale toothed-bird collections (R. C. Fox, personal communication).

Meinke *et al.* attribute the undeniably close resemblance of dentinal rings in dinosaurs and crocodiles to phylogenetic propinquity. *Champsosaurus*, a Late Cretaceous–Eocene eosuchian (and undoubted ectotherm), displays dentinal rings like those in Upper Cretaceous dinosaurs and crocodiles. Would Meinke *et al.* explain this in terms of 'phylogenetic propinquity' of archosaurs and eosuchi-ans, groups whose last common ancestry is no younger than Permian and perhaps Carboniferous¹³ (almost as remote as for archosaurs and mammals)?

Phylogenetic 'explanations' only describe results of process, not process itself (at least for those who believe natural selection is the source of adaptive resemblance between organisms), and it is clearly self-contradictory that resemblances purported to stem from phylogenetic relationship would have completely different physiologic origins, as Meinke *et al.* would have us believe.

Finally, Meinke *et al.* argue that, owing to their diversity, dinosaurs might be expected to show a range of physiological thermoregulation. Perhaps, but if so diversity by itself will not carry the argument: after all, Recent reptiles are far more diverse taxonomically than are dinosaurs, yet all are ectothermic.

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Feroxyhyte on Mars?

BURNS discussion¹ on the possibility of the mineral feroxyhyte occurring on the surface of Mars is potentially misleading. In the original description of this mineral Chukhrov *et al.*², following Feitknecht³, distinguished between a strongly magnetic phase, δ -FeOOH, and a "practically nonmagnetic" phase δ' -FeOOH. It was the weakly or nonmagnetic δ' phase to which the mineral name feroxyhyte was applied. Burns seems to have equated the two phases and uses data in support of his hypothesis most of which are derived from the synthetic δ -FeOOH. The difference may be of more than semantic significance because the ferromagnetic, deep brownish δ -FeOOH is formed by the vigorous, rapid oxidation and topotactic replacement of Fe(OH)₂. As this is a process unlikely to occur in natural conditions at the Earth's surface, δ -FeOOH has yet to be reported as a mineral. On the other hand, δ' -FeOOH (feroxyhyte), while also requiring the topotactic replacement of precipitated Fe(OH)₂, is obtained by a slower oxidation and is a yellow-brown ochreous material when dried². This is the mineral reported from marine sediments and gley soils². The yellow-brown colour is almost identical to the description of the soils at the Viking Lander sites⁴. However, if feroxyhyte is nonmagnetic then its major presence on the weak magnets attached to the Viking Lander backhoes⁵ is improbable. Chukhrov *et al.* noted that some of the δ' -FeOOH samples they studied contained admixed maghaemite (γ -Fe₂O₃) which they felt contributed to the weak magnetism of these individual samples. Thus, on the basis of colour, feroxyhyte (δ' -FeOOH) might indeed be a constituent of the surface of Mars and the magnetic experiments may have picked up admixed maghaemite⁵, but the possibility of δ -FeOOH on Mars seems remote.

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BURNS REPLIES—When it was suggested that feroxyhyte might occur on the surface of Mars¹, I was well aware^{2,3} of the claimed distinction between the ferromagnetic phase δ -FeOOH and the terrestrial mineral feroxyhyte (δ' -FeOOH). Towe points out that in their original descriptions of feroxyhyte, Chukhrov *et al.* thought the weak magnetism in some δ' -FeOOH samples was due to maghaemite (γ -Fe₂O₃) impurities^{4,5}. Note, however, that synthetic δ -FeOOH phases display variable saturation

magnetization^{6–13,17} as a result of particle size variations, implying the coexistence of δ -FeOOH and δ' -FeOOH in most synthesis products. Maghaemite, too, shows similar tendencies towards superparamagnetism^{14–16}. Towe has focused his attention on the magnetic properties of δ -FeOOH- δ' -FeOOH assemblages without commenting on other favourable properties of them relevant to martian geochemistry. I contend that feroxyhyte δ' -FeOOH- δ -FeOOH admixtures must be reckoned as a prime candidate for the colour, chemisorption, spectral, redox, paragenesis and magnetic properties of the surface of Mars.

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Anisotropy of Young's modulus of bone

The Hashin-Rosen model was recently applied by Katz¹ to bone to explain the variation of Young's modulus, E , for bovine femoral specimen with respect to the orientation of specimen axes cut at the angle ϕ to the long axis of the bone. From Fig. 4 of Katz's report it can be seen that (1) in the case of dry bones E fell from 20 GN m⁻² at 0° to 13 at 10°. At 20°, 50°, 60° and 70° E was 13. Only at 30° and 40° was it 14; (2) in the case of wet bone E was 17 GN m⁻² at 0° then fell steadily to 10 at 50°. E then increased to 14 at 60°, remained steady at 14 up to 70° and thereafter gradually declined to about 11 GN m⁻².

If values of E for the dry and wet bone specimens are considered to be significantly different, water must have an important role in determining the mechanical properties of bone. The Hashin-Rosen model does not take this role into account.

If, on the other hand, values of E for the dry and wet bone specimens are not considered to be significantly different, E values between 10° to 80° lie between 13 and 14 GN m⁻², that is, E does not seem to be affected by ϕ . This result is contrary to the Hashin-Rosen model. Accordingly, I wonder whether the Hashin-Rosen model can be profitably applied to bone.

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1. Katz, *Nature* **283**, 106 (1980).

KATZ REPLIES—Further explanation seems necessary for the role of the Hashin-Rosen calculation in modelling the elastic properties of bone. This is the structural composite phase of the hierarchical modelling. It cannot *per se* account for the effects of wet compared with dry bone. This entails considering the material composite nature of the system during the hierarchical modelling.

First, as I stated¹, dry bone is stiffer and more rigid than the same specimen when wet. This is attributed to changes in the elastic properties of the organic matrix of bone. Second, the Young's modulus, E , of bone does vary with angle ϕ as shown by many experimenters for haversian bone, for so-called plexiform bone and for mixed specimens. The bovine bone specimens used by Bonfield and Grynpas² probably included all three kinds; unfortunately, they reported no structural observations. Indeed, this might explain why some wet specimens were stiffer than some dry ones (plexiform bone is stiffer than haversian bone).

These facts can be incorporated in the Hashin-Rosen calculation; values of E_{GS} , ν_{GS} , E_{ost} and ν_{ost} can be adjusted. Thus, $E(\phi)$ in Fig. 3 of my report is approximately 30% greater than the equivalent $E(\phi)$ on Fig. 4. Also, $E(\phi)$ at any angle ϕ can vary as much as 80% (see Fig. 4 of ref. 1) simply by altering the percentage of longitudinal osteonic collagen fibres. Thus, curve *a* of Fig. 4 simulates dry bone, and curves *b*, *c* and *d* in effect simulate wet bone behaviour. In reality, this calculation accounts for the effects of sample variation described above, that is, more longitudinal fibres, and thus stiffer lamellae, so simulating plexiform bone.

Unfortunately, my original report¹ could not be expanded to discuss all this; a longer, more complete paper is being prepared. Finally, a hierarchical model wherein a structural composite is superimposed on a material composite does simulate the elastic properties of bone.

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BOOK REVIEWS

Environmental issues: how much science, how much politics?

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THE 1970s could well be remembered as the decade of the environment, a decade that was ushered in by warnings of impending disaster. But it was also a time of controversy. Opinions differed widely both on the nature of the threat to the environment and on its causes and cures. During the decade, the focus of attention shifted from pollution to a growing concern over the exhaustion of fossil fuels and resources. The period also saw the emergence of a more radical environmentalist movement, impatient with mere cosmetic meliorist strategies, arguing that the roots of the environmental crisis lie deep in the structure and values of contemporary society, and that no mere technical fix can solve the problem. Now, in the 1980s, nuclear power looks set fair to become the focus for continuing radical protest.

The controversies spawned a vast literature. Any attempt to master the thousands of articles and books and to achieve some kind of diagnosis and stock-taking is a daunting prospect. These two books, however, are both impressive efforts to generate an interpretative framework. Together with Tim O'Riordan's earlier study (*Environmentalism*; Pion, 1976), they constitute a guide through the massive literature and complexities of the issues.

One conclusion is inescapable. Environmental controversies go far beyond scientific and technical debate about reserves of non-renewable resources, methods for safe disposal of radioactive waste and the "risks" of various methods of generating energy. Pollution, for example, is not a scientific term: it conflates a value judgement. In the words of Mary Douglas, pollution is dirt in the wrong place. Similarly, "safe" systems always entail a judgement about an acceptable level of hazard. Behind controversies about low-level radiation, the routing of a motorway or optimum levels for the control of pollution, there is a hidden agenda involving the increasing powerlessness of individuals to influence decisions affecting their lives and the question of the extent to which economic growth should be at the expense of other values.

There are, of course, other blatant and obvious interests underlying the protest of those who do not want a motorway through their own back gardens or a hydro-electric scheme that might spoil their

Environment, Ideology and Policy. By Francis Sandbach. Pp.254. (Basil Blackwell/Allanheld, Osmun: 1980.) Hardback £12.50, \$26.50; paperback £4.95. *The Environment from Surplus to Scarcity.* By Allan Schnaiberg. Pp.464. (Oxford University Press: 1980.) Hardback \$14.95; paperback \$9.95, £6.75.

fishing. But beyond this, environmental policies are essentially political in the sense that the interests of some are safeguarded, while those of others are threatened. The central thesis of Sandbach's book is that it is political ideologies that mould both policy and action.

This point is not easily taken by many who see the debate over nuclear risk, for example, as essentially factual. For them it is a question of an objective analysis of degrees of risk for various sources of energy as the basis for a rational decision to choose those methods which the calculus shows to be least risky. From this perspective the rejection of nuclear power is at best ill-informed, and at worst emotional and irrational. But there is a hidden agenda on both sides of the debate. Both are essentially rooted in the alternative ideologies which Stephen Del Sesto has discussed (*Public Policy* 28 (1); 1980); both come to conclusions which are perfectly sensible and reasonable within their respective frameworks of meaning. The problem is to unpack the debate in such a way that the hidden beliefs and values of both sides are made explicit. Even this will not go far enough. Each side is convinced of the self-evident logic of its own position. In short, the debate is embedded in competing doctrines about the nature of society, and above all, about what are the overriding values that policy should seek to maximize.

Despite the central role which the notion of ideology plays in Sandbach's analysis, nowhere does he unpack the concept. It is necessary to distinguish at least two senses in which the term is used. First, there is the argument that ideology refers to knowledge which is distorted by the interests of different groups. In order to substantiate this usage, it would be necessary to demonstrate ways in which scientific knowledge — of the effects of low-level radiation, for example — is

actually related to interests. Of course, it is easy to show that a substantial proportion of the total scientific effort is related to production and defence. The other side of the coin is to demonstrate that much research does not get done because it is not in the interests of dominant interest groups. Schnaiberg's analysis has essentially the same message: that it is the distribution of economic and political power which shapes the kind of research which does get done. So, argues Schnaiberg, there has been a major emphasis on production research to the relative neglect of research on environmental impacts. But he is more convincing than Sandbach in presenting supporting evidence. Equally important, he provides evidence of the subtle (and not so subtle) pressure in the USA against the communication of evidence contrary to powerful interests. Consequently, he argues, society simply lacks the necessary scientific intelligence for realistic environmental policies.

There is a second sense in which it is possible to distinguish between science and ideology. Whenever knowledge and beliefs are used to justify and legitimize policies and courses of action, science is being put to ideological uses to support particular interests. This does not necessarily mean that the knowledge is distorted by interests. It simply draws attention to the distinction between knowledge and its uses. So when scientists give evidence at public inquiries, they are, willingly or not, caught up in political debate. This may help to explain the lack of confidence and trust in science among environmentalists. The achievements of science in modern industrial societies underlie the generally high respect for science and scientists. To appeal to expert opinion is a powerful weapon in political debate. But to sustain respect, science must be seen to be impartial and objective; it is this that many environmentalists doubt. Interestingly, the appearance of scientists at inquiries has contributed to the erosion of confidence. It has increased awareness of the extent of disagreement: on almost all major issues, expert opinion can be ranged on both sides of debate.

The two books are especially critical of the claims of such techniques as cost-benefit analysis, technology assessment and environmental impact analysis to be objective and scientific. The great

weakness, say both Sandbach and Schnaiberg, is that such approaches fail to investigate the original basis for technological choice. Much of the debate over nuclear power, for example, has focused on its social and environmental consequences without asking what, for Sandbach, is the fundamental question: what are the interests which promote this form of energy? Only by uncovering the hidden agenda can there be a debate about nuclear power which comes to grips with the fundamental issues.

Both books challenge the notion of technological determinism — technology as some independent and impartial force, there being no escape from its imperatives — and explore the ways in which technology is shaped by the economic and political system. Sandbach gives a particularly interesting and thought-provoking account of technology and the environment in China. Chinese economic policy would not be judged efficient in market economy terms precisely because it has sought to incorporate non-economic political goals, such as rural self-sufficiency, worker management of factories, and protection of the natural environment and resources. And it has deliberately sought to develop technologies which are appropriate to the achievement of such goals. This has not, incidentally, been without its environmental problems, notably that of organic pollution in the rural areas.

Sandbach and Schnaiberg, then, make a powerful case for the view that environmental policy goes far beyond technical issues. The way a society treats its environment is rooted in the fundamental structure and values of that society. Science and technology, too, are functions of social systems.

While both books attempt to explore the implications of different political positions for the environment, both are more optimistic about the ability of the left to find benign solutions avoiding the more extreme forms of centralized state control which scarcity may generate. While Schnaiberg manages to avoid obvious partiality, Sandbach is not so successful. He is less than convincing, for example, about the possibility of an "objective" Marxist science, free from political and ideological distortions.

Both books are scholarly and well researched. Both carry the same essential message — that science and technology function within a political context. Few scientists and even fewer technologists will like this. By education and inclination, many would prefer a de-politicized world in which policy is determined by "facts" and expertise. Environmental controversies, however, supply convincing evidence to the contrary. □

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At the core of chemical catalysis

F. R. Hartley

Homogeneous Catalysis: The Applications and Chemistry of Catalysis by Soluble Transition Metal Complexes. By George W. Parshall. Pp.240. (Wiley-Interscience: 1980.) £14.90, \$35.

THIS book by Dr G. W. Parshall, Director of Chemical Science at du Pont, aims to provide a balanced description of homogeneous catalytic reactions. In this it succeeds admirably. There are few people better qualified to write such a book and everyone involved in the field, student or research worker in an academic or industrial laboratory, will be grateful to Dr Parshall for wading through the enormous literature of homogeneous catalysis to expose the real core of the subject to close scrutiny.

After two brief, but valuable, introductory chapters there follow nine chapters on homogeneously catalysed chemical reactions. All the major industrial processes that use homogeneous catalysis are covered, as well as the more useful laboratory syntheses. Clearly, some selection has been necessary here, but in my judgement it has been well done. The final chapter looks to the future, considering the known as well as likely developments in feedstocks as well as possible future developments in catalysis, including supported metal complex catalysts.

The strengths of this book are many. Foremost amongst them is the air of industrial reality that pervades the whole book. This is beautifully illustrated by the comment that the intensive study of homogeneous olefin hydrogenation catalysts seems anomalous because soluble catalysts are rarely used either in industrial or laboratory hydrogenations. Many of the chapters end with a number of important general references to guide the reader into the wider literature. Key references in the text have been chosen with great care.

This book is one that everyone working in the field of homogeneous catalysis will want to have close to hand, and lecturers will want several copies available in the library for their students. The book summarizes what is known and where the applications of the subject lie; it emphasizes areas in which more research is needed to increase our understanding of the mechanisms of homogeneously catalysed reactions; it provides pointers to the future. I recommend it unreservedly to anyone starting or established in the fields of synthetic, industrial and catalytic chemistry. □

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New ideas about parasites

Donald R. Strong

Evolutionary Biology of Parasites. By Peter W. Price. Pp.237. (Princeton University Press: 1980.) Hardback \$22, £9.70; paperback \$8.75, £3.80.

PRICE'S thoughtful and ambitious little book is an ecumenical overview which considers phytophages, parasitoids and hemiparasitic green plants, as well as the subjects of classical parasitology, as functional analogues. Such general perspectives have not been of great interest to parasitologists, who are more concerned with details of the taxonomy, life-cycles and pathology of the myriad pests of animals and plants. Price, in contrast, is an ecologist and evolutionist, and as such works in an alternative tradition of broad generalization and synthesis. I hope that this does not deter mainstream parasitologists. Ecologists will read the book; the author's reputation and that of the *Princeton Monographs in Population Biology* ensure this. But biologists who

work on specific parasitic groups have much to contribute to an eco-evolutionary synthesis in parasitology; and much to gain from the book. Ultimate success for Price's efforts towards a synthesis will come only from a rapprochement of these two camps.

After an introduction, chapters on general concepts, non-equilibrium ecology and evolution, genetics, radiation and host-specificity, niches and orthodox community theory, and parasite influences on host evolution follow. Finally there is a coda of ideas for further study.

The most interesting chapter to me is that on communities. Here, Price represents the new academic ecology well, in questioning the basic assumptions of the old, ideal Hutchinsonian school. The most important assumption of the idealists is, of course, that communities are primarily a product of the competitive interactions among their component species. Price meets this head-on with Table 6.1, which shows that the literature indicates non-

interactive coexistence to be quite common among species of gut parasite. In summary he says: "There is little evidence to suggest that competition has been an organizing force [for many parasites]". K. Rohde's important overview of species interaction among fish gill parasites was published after this book went to press (*Am. Nat.* 114, 648; 1979), but his earlier work is reviewed. For gill parasites, Rohde comes to a conclusion similar to Price's; so do John Lawton and myself for the phytophagous insects, in a paper now in press (*Am. Nat.*).

Clearly, exceptions to this generalization exist in both zoophagous and phytophagous groups; interspecies competition does sometimes affect coexistence. But the great preponderance of cases involve loose organization (if any) among parasitic species at similar trophic levels, with no substantial shift from fundamental to realized niche in the presence of another species.

I like Price's book so much that I feel entitled to make two suggestions as to how it might be improved. First it is unfortunate that "community interaction" is treated only in the purblind Hutchinsonian sense, which studies competition to the virtual exclusion of all else. Interactions can occur horizontally in food webs — as competition or mutualism among species at very similar trophic levels — and they can occur vertically as predation and parasitism from above, or through factors such as the noxious and nutritious aspects of hosts as food from lower in the web. Perhaps this omission was made from modesty; Price has published much about community influences of natural enemies on phytophagous insects.

My second reservation concerns "coevolution", a term which here has as many references in the subject index as any other process. Price deals critically with most conceptual flabbiness in ecology and evolution, but he is soft on coevolution. For example, specialization of parasites is discussed as if it were necessarily a coevolutionary process — need it be? In another example, the inverse relationship between host breadth of agronomid flies and the diversity of their hosts' phytochemicals is treated as if it were a necessarily coevolutionary phenomenon. Again, need it be? Coevolution implies more than just the evolution of two species that live together. It implies reciprocity of influence. Evolutionary influence of host upon parasite can occur without any influence being returned, especially with benign parasites living in a non-equilibrium world.

But this is niggling. Price's book is exciting, imaginative and its influence might even be enduring, if parasitologists and eco-evolutionists find common interests as its result. □

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Developments in geodetic theory

William M. Kaula

Advanced Physical Geodesy. By H. Moritz. Pp.500. (Abacus: Tunbridge Wells, UK/International Scholarly Book Services: Oregon, 1980.) £26, \$57.

THIS treatise concerns developments in the theory of "classical" physical geodesy that have taken place since the publication of *Physical Geodesy* by W. A. Heiskanen and H. Moritz (W. H. Freeman, 1967). The implication of "classical" is that the figure and gravitational field of the Earth are considered as constant in time. The book is intended for research workers and graduate students in geodesy and gravity, and opens with a thorough introductory exposition on the background to the subject. About half the book is devoted to advanced least-squares collocation theory and one-third to the geodetic boundary-value problem. A topic not treated is the differential geometry of the gravitational field; for this *Mathematical Geodesy* by M. Hotine (US Dept of Commerce, 1969) is still the best book available.

The strengths of the book lie in the soundness of its mathematical foundations, the clarity of the writing and the authoritative tone of its discussion of modern developments in geodetic theory. Despite the rigour of the mathematics the text has an easy style, understandable to engineers and physical scientists. The authoritative tone arises naturally from Helmut Moritz being a leading contributor to the field, but work by others, such as Molodenskiy, Krarup, Bjerhammar and Sanso, has been well integrated.

To me the weaknesses of the book all appear to be in the area of least-squares collocation: firstly, an excessive simplification of gravity field properties, leading to an under-estimation of some difficulties; and secondly, a failure to take advantage of concepts and techniques developed outside geodesy.

The principal simplification is that gravity is taken to be homogeneous and isolated: a global covariance curve is taken as given, and no cross-covariance with other variables, such as topography, is considered (although accounting for topography by isostatic reduction is mentioned). However, it is in these areas that the greatest difficulties occur in applying collocation: the appropriate data selection to determine the covariance curve, and how to take into account topography as an influence on the distribution of data, as well as a correlate, and hence predictor, of gravity. Although Moritz recognizes that complete representation of a continuum like gravity requires an unlimited number of parameters, he does not present tools like singular value analysis or the resolution

matrix to select the limited parameter set which can be determined from a finite data base. The discussion of "improperly posed problems" does not bring out the idea of "trade-offs" between accuracy and resolution, and the heuristic opportunity of comparing similar inference problems in different fields is not taken.

Moritz's treatise is essential for any researcher specializing in physical geodesy. However, for the non-geodesist wishing to obtain some insight about the nature of the gravity field and the methods applied to it the 1967 text by Heiskanen and Moritz seems preferable. □

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Causal dilemmas

P.E. Bryant

Social Interaction and Cognitive Development in Children. By Anne-Nelly Perret-Clermont. Pp.206. (Academic: 1980.) £14.80, \$34.

No subject is going to solve its problems until it realizes how difficult those problems are. A poignant example can be found in child psychology, which is faced with the major difficulty which it does not recognize and is certainly nowhere near to solving.

The problem is one of causes. Children's behaviour changes as they grow older, and it is easy enough to say what these changes are. But what makes them happen? What are the causes of, for example, intellectual development? Child psychologists have typically relied on quite inadequate techniques for answering this kind of question. An obvious example is their use of correlation. To find, as someone did, that the more a father talks to his children at breakfast the brighter they are, is, in child psychology, to conclude that parental chatter determines intelligence. But what about the possibility, *mutatis mutandis*, that brighter children provoke the tired parent into talking while dim children do not?

Psychologists have one other major technique for tracking the causes of development, and that is the training experiment, which also has weaknesses. These weaknesses are to be found in the work described in Perret-Clermont's book which attempts to demonstrate that social interactions in the form of discussions and arguments between children are an

important factor in causing intellectual changes.

The rationale behind the training experiment is simple. You think that social interaction is important, so you give a child a pre-test on some important cognitive skill like Piaget's conservation test, then give them a training period, and then a post-test exactly like the pre-test. If they are better on the post-test than a control group, you conclude that social interaction normally causes the development in question.

There are two difficulties, neither solved by Perret-Clermont. The first is that these positive results might be quite artificial. Many other people have done successful conservation training experiments using quite different methods. There must be at least a dozen successful ways, most of which do not involve social interaction between children. There is no way of knowing which has isolated the real cause.

Secondly, it's peculiarly difficult to get the controls right. For example we cannot say from Perret-Clermont's data whether it is social interaction with anyone or social interaction with children that is important. She does not take her effects apart. Nevertheless her work is simple and ingenious, and she presents an attractive causal hypothesis. But how are we ever to test it properly? □

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Vesicle in a basket

Ernst Ungewickell

Coated Vesicles. Edited by C.D. Ockleford and A. Whyte. Pp.344. (Cambridge University Press: 1980.) £40, \$90.

FOR more than 10 years, up to about 1975, the study of coated vesicles was almost exclusively the province of morphologists who early on had recognized the importance of these organelles in exo- and endocytotic processes. This state of affairs changed dramatically when B. Pearse succeeded in isolating pure vesicles in quantity, and thus made them available to biochemists. It is this latter group who will profit most from this monograph.

The volume contains contributions by representatives of a number of specialized fields, most of whom offer stimulating discussions and speculations on what are often rather fragmentary data. The 12 chapters fall into three groups: an introductory survey of the field; exploration of specific functions of the vesicles; and discussions of models aimed at describing endocytotic events at a molecular level.

The book opens with a classification of

endocytotic processes, and an evaluation of the role of coated vesicles in micropinocytosis and their comparison structurally and functionally with smooth-walled micropinocytotic vesicles. This is followed by a summary of the occurrence of coated vesicles in different species and cell types, and a discussion of the different pathways of intracellular transport.

The logical sequence of the contributions proceeds with a consideration of the coated vesicle in plant cells. Subsequent chapters describe in detail the involvement of coated vesicles in IgG transmission, their role in membrane retrieval in neurosecretory cells, and their function in vitellogenesis and secretory processes. All of these reviews are supplemented with high-quality electron micrographs showing the characteristic appearance of coated vesicles from different sources in both median and tangential sections.

Molecular aspects of receptor-mediated transport are discussed next, and models proposed by various researchers are compared. The authors of this contribution

conclude that specific protein transport is too complex a process to be described by any one of the proposed models. It is my impression that the same might be said of the models which try to explain how membranes invaginate to assume the configuration of coated vesicles, a topic which is also discussed. In the penultimate chapter, structural aspects of coated vesicles are reviewed; this reveals that surprisingly little work has been done on the structure of the major coat protein, clathrin. The book concludes with a cautious discussion of the importance of coated vesicles in medical science.

In summary, the book provides comprehensive coverage of the knowledge of coated vesicles up to the end of 1978 (a list of more recent publications appears in an appendix) and clearly indicates directions for future research. I recommend it to anyone who is new to the field or who is considering working with coated vesicles.

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Much to ponder in lipid evolution

T.W. Goodwin

Lipids in Evolution. By William R. Nes and W. David Nes. Pp.195. (Plenum: 1980.) \$29.50, £18.59.

THE authors of this book, *père et fils*, are clearly fascinated by the problem of relating biochemical parameters to the classical ideas on evolution. Concentrating on lipids, they have sifted through a great amount of information and bravely attempted to discern clear evolutionary patterns. A perusal of their final chapter indicates how difficult this data analysis was and how few new correlations have emerged. In almost every aspect studied, facts crop up which invalidate an emerging generalization. Indeed the authors' occasional frustrations are revealed by such comments as "The situation is made worse by the phylum Mollusca". This follows the view that as arthropods cannot synthesize sterols it would be reasonable to assume that molluscs, which from fossil records pre-date arthropods, would also not synthesize sterols; alas, "biosynthesis of sterols is a common property of molluscs".

The first four main chapters deal succinctly with dating and chronology, the palaeontological record, the origin of oxygen, and the temperature and pH problem. It is clear from the chapter on oxygen that anaerobic organisms can make some unsaturated fatty acids and isoprenoids anaerobically from acetyl-CoA. A discussion of how anaerobic

organisms make acetyl-CoA would have been most welcome, as would some comment on the quantitative aspects of acetyl-CoA, lipid and isoprenoid production in the presence and absence of oxygen in organisms such as yeast.

"Phylogenetics and Occurrence", the next chapter, is a straightforward and thorough account of lipid and sterol distribution in nature. The main conclusion is that no lipid distribution is unique to prokaryotes or to eukaryotes, and this is emphasized by the observation that the fatty acid pattern in blue-green bacteria (algae) is much closer to that of the eukaryote algae than to that of the photosynthetic bacteria.

The authors next turn to a consideration of phylogenetics and biosynthesis, paying particular attention to the important bifurcation in sterol synthesis at the point of cyclization of squalene 2,3-oxide. This clearly separates non-photosynthetic from photosynthetic organisms. In the former, the first cyclization product is lanosterol, whereas in the latter it is cycloartenol. The reason for this bifurcation is still obscure.

This is a book packed with interesting information and spiced with enjoyable speculation. It will help to broaden the outlook of conventional natural-product chemists and will give biologists much to ponder over. □

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